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Classification of *Colletotrichum lindemuthianum* races in differential cultivars of common bean

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ABSTRACT. Anthracnose caused by the fungus *Colletotrichum lindemuthianum* is one of the main diseases affecting the common bean (*Phaseolus vulgaris* L.), and the pathogen is characterized by wide variability, with more than 50 physiological races identified in Brazil. Greater occurrences of races 65, 73, and 81 have been observed in Brazil along with the occurrence of pathogenic variability among isolates of a single race, destabilizing the resistance of commercial cultivars. Therefore, the aim of this study was to identify physiological races of *C. lindemuthianum* isolates collected in the states of São Paulo and Santa Catarina, Brazil and to test for variability among the isolates of race 65. The classification of 51 isolates resulted in the identification of 10 different physiological races: 4, 38, 55, 65, 73, 81, 83, 85, 321, and 351. Races 65 and 81 predominated, with frequencies of 37.25 and 35.29%, respectively. Regarding the isolates of race 65, wide physiological variability was evident, suggesting that a new differential set should be applied to detect the levels of variation among isolates of a single race of the pathogen.

Keywords: physiological variability, levels of variation, anthracnose.

Classificação de raças de *Colletotrichum lindemuthianum* em cultivares diferenciadoras de feijoeiro comum

RESUMO. A antracnose causada pelo fungo *Colletotrichum lindemuthianum* é uma das principais doenças do feijoeiro comum (*Phaseolus vulgaris* L.), o patógeno caracteriza-se por apresentar uma grande variabilidade, com mais de 50 raças fisiológicas identificadas no Brasil. Tem-se evidenciado a maior ocorrência das raças 65, 73 e 81 no país e também a ocorrência de variabilidade patogênica entre isolados de uma mesma raça, que desestabilizam a resistência das cultivares comerciais. Portanto, o objetivo com este trabalho foi a identificação de raças fisiológicas de isolados de *C. lindemuthianum* coletados no Estado de São Paulo e Santa Catarina, Brasil e, detectar a ocorrência de variabilidade patogênica entre isolados pertencentes à raça 65. A classificação de 51 isolados resultou na identificação de 10 diferentes raças fisiológica: 4, 38, 55, 65, 73, 81, 83, 85, 321 e 351, com destaque para a raças 65 e 81, que apresentaram uma frequência de 37,25 e 35,29%, respectivamente. Com relação à avaliação entre os isolados da raça 65, ficou evidente a alta variabilidade dos mesmos, sugerindo a utilização de um novo conjunto diferenciador para detectar os níveis de variação entre isolados de uma mesma raça do patógeno.

Palavras-chaves: variabilidade fisiológica, níveis de variação, antracnose.

Introduction

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc & Magn.) Scribner, is one of the main diseases affecting the common bean (*Phaseolus vulgaris* L.), and the disease may occur in all crop seasons (dry, winter, and rainy seasons), causing lower yield and grain quality. The crop may suffer losses of up to 100% when the infection occurs at the beginning of the cycle along with low temperatures and high relative humidity (Dalla Pria & Silva, 2010). The most effective means to control the pathogen is using resistant cultivars, though the wide physiological variability of the pathogen may break down the resistances of commercial cultivars (Davide & Souza, 2009).

Approximately 50 physiological races of the pathogen have been identified in Brazil: 1, 5, 7, 8, 17, 23, 31, 55, 64, 65, 67, 69, 71, 72, 73, 75, 77, 79, 81, 83, 85, 86, 87, 89, 93, 95, 96, 97, 101, 102, 105, 109, 111, 117, 119, 121, 123, 125, 127, 137, 193, 217, 249, 320, 321, 337, 339, 343, 453, and 585, but races 65, 73, and 81 have been identified with greater

frequency due to their aggressiveness (Silva, Souza, & Ishikawa, 2007).

Physiological races are identified following Pastor-Corrales (1991) by evaluating the resistance response of twelve differential cultivars; however, using these cultivars has not been effective in determining the variability exhibited by the pathogen.

This deficiency in detecting the variability of the species was also seen by Davide and Souza (2009), suggesting that the pathogen race diversity is greater than currently known. Souza, Camargo Júnior, and Pinto (2010) indicated that certain cultivars are resistant to certain isolates and susceptible to others of the same race of the pathogen, suggesting variability within races and making the introduction of resistance in new common bean genotypes even more difficult. This situation was also observed by Ishikawa, Ramalho, and Souza (2011), who suggested a new group of differential cultivars to detect variation among isolates of race 65 of *C. Lindemuthianum*. This high variability shows the difficulty in controlling the disease and the importance of identifying new resistance genes.

The aim of this study was to classify the physiological variability of 48 *C. lindemuthianum* isolates collected in the state of São Paulo and 3 collected in the state of Santa Catarina as well as to classify the levels of physiological variability among isolates of race 65.

Material and methods

To obtain *C. lindemuthianum* isolates, tissues of symptomatic plants were collected from different common bean production regions in São Paulo and Santa Catarina State, Brazil.

Each sample was handled in the Plant Health Research and Development Center laboratory of the Agronomic Institute - IAC (Campinas/São Paulo State, Brazil), where direct isolations from infected organs (leaf, stem, or pod) were made followed by monosporic cultures. After obtaining the monosporic cultures, the isolates were transferred to new oatmeal agar media and kept for 10 days in a BOD at 24°C and in absence of light for spore production.

The physiological variability of the isolates was classified according to the evaluated resistance response to *C. lindemuthianum* in twelve differential cultivars using the binary value system suggested by Pastor-Corrales (1991), listed in Table 1.

The 12 differential cultivars and two susceptible controls (Rosinha G_2 and Pérola) were inoculated at the vegetative stage (V_2) by spraying the inoculum at concentrations of 1 x 10^6 conidia mL^{-1} over the entire shoot surface area. Then, the seedlings were kept in a growth chamber for 48 hours at a controlled temperature (20°C) and 95% relative humidity using a nebulizer with a 12 hours photoperiod. The humidity chamber was then turned off and the seedlings remained for 10 more days at 24°C. Symptoms were evaluated through summing the binary values of the differential cultivars susceptible to each isolate tested (Table 1).

Table 1. Classification of physiological races of *Colletotrichum lindemuthianum* according to the reaction of twelve differential cultivars of the common bean (Pastor-Corrales, 1991).

Order	Differential variability	Binary value*
1	Michelite[b]	1
2	MDRK[a]	2
3	Perry Marrow[a]	4
4	Cornell 49242[b]	8
5	Widusa[a]	16
6	Kaboon[a]	32
7	México 222[b]	64
8	PI 207262[b]	128
9	TO[b]	256
10	TU[b]	512
11	AB 136[b]	1024
12	G 2333[b]	2048

*The designation of races is obtained by summing the binary values of the differential cultivars susceptible to the pathogen. [a]Andean genotypes; [b]Mesoamerican genotypes.

To evaluate the variability within race 65, we adapted the methodology of Ishikawa et al. (2011), who suggested using eight differential cultivars for isolates collected in the state of Minas Gerais (with their respective binary values): BRS Estilo (2^0), BRSMG Majestoso (2^1), BRS Supremo (2^2), BRSMG União (2^3), BRS Valente (2^4), Ouro Vermelho (2^5), BRSMG Madrepérola (2^6), and BRSMG Talismã (2^7). However, for the isolates evaluated in this study collected from São Paulo and Santa Catarina States, it was necessary to attribute binary values to the Ouro Negro (2^8), BRS Cometa (2^9), and BRS Esplendor (2^{10}) cultivars (Table 2).

Table 2. Classification of isolates of race 65 of *Colletotrichum lindemuthianum* based on the methodology proposed by Ishikawa et al. (2011).

Order	Differential Variability	Binary Value
1	Ouro Negro	2^8
2	BRSMG Majestoso	2^1
3	Pérola*	-
4	BRSMG Talismã	2^7
5	BRS Valente	2^4
6	BRSMG Madrepérola	2^6
7	BRS Supremo	2^2
8	BRS Estilo	2^0
9	BRS Cometa	2^9
10	BRS Esplendor	2^{10}
11	Ouro Vermelho	2^5
12	BRSMG União	2^3

*Control of Susceptibility. The designation of variability within race 65 of the pathogen is obtained by the summing the binary values of the differential cultivars susceptible to the isolate tested.

Thirteen isolates previously classified as race 65 originating from samples collected from São Paulo and Santa Catarina States were evaluated: 14779 and 14780 (Botucatu - São Paulo State), 14781 (Jaú - São Paulo State), 14790 (Campos Novos – Santa Catarina State), 14782 and 14783 (Botucatu - São Paulo State), 14784 and 14834 (Campinas - São Paulo State), 14789 (Mococa - São Paulo State), 14835 (Campinas - São Paulo State), 14788 (Capão Bonito - São Paulo State), 14652 (Cerqueira César - São Paulo State), and 14670 (Iguaçu - São Paulo State). The variability within this race was evaluated by summing the binary values of the differential cultivars susceptible to each isolate tested.

Results and discussion

Ten physiological races of *C. lindemuthianum* were identified as races 4, 38, 55, 65, 73, 81, 83, 85, 321, and 351 in São Paulo State, and one was identified (race 65) in the three isolates collected in Santa Catarina State (Table 3).

According to Carbonell et al. (1999), the main races of the pathogen found in São Paulo State were 31, 65, 81, and 89. In Santa Catarina State, according to Gonçalves-Vidigal, Silva, Vidigal Filho, Gonela, and Kvitschal (2007), race 65 was the most frequent, identified in 11 of the 32 isolates evaluated. Those results agree with the results obtained in this study, in which races 65 and 81 were the most frequent among the 51 isolates classified (Table 3). Nineteen of the 51 isolates were classified as race 65 and 18 isolates as race 81 with a frequencies of 37.25 and 35.29%, respectively. These two races were the most aggressive to the crop and are widely disseminated throughout all common bean producing areas in Brazil.

This dissemination is facilitated by the free trade of grains among the states and because many producers reuse the grains previously produced in the same growing area, thus increasing the potential of the inoculum from one crop season to another and favoring the pathogenic variability of *C. lindemuthianum* (Talamini et al., 2004).

Races 4, 38, 55, 83, 85, 321, and 351 were found with lesser frequency, and races 4, 38, 321, and 351 were classified only once among the 51 isolates. Races 38 and 351 had not previously been reported in Brazil. Gonçalves-Vidigal, Thomazella, Vidigal Filho, Kvitschal, and Elias (2008) also identified races 67, 83, 101, 103, 105, and 581 for the first time in the state of Santa Catarina, showing the wide variability and dissemination of the pathogen.

Table 3. Classification of physiological races of *Colletotrichum lindemuthianum* isolates.

Isolate	Location	Race	Isolate	Location/ São Paulo State	Race
4759-1	Capão Bonito São Paulo State	38	14829	Avaré	*
4759-2 (5)		38	14830	Araras	*
4759-3 (1)		38	14831	Colina	321
9253	Piracicaba/ São Paulo State	65	14832	Pindorama	*
9876-3		83	14833	Itapenitinga	*
9876-5		85	14834	Campinas	65
9876-6	Campinas São Paulo State	81	14835		65
14244		4	10885-2	Taquarituba	73
14245		38	10885-3		73
14246		55	13039-2	Capão Bonito	65
14648	Avaré/ São Paulo State	*	13039-4	Capão Bonito	81
14649	Cerqueira César/ São Paulo State	*	13160-1	Monte Alegre do Sul	81
14650	Avaré/ São Paulo State	351	13160-3	Monte Alegre do Sul	65
14652	Cerqueira César/ São Paulo State	65	13160-4	Monte Alegre do Sul	65
14670	Iguaçu/ São Paulo State	65	13348-1	Capão Bonito	81
14778	São Manuel/ São Paulo State	81	13348-2	Capão Bonito	65
14779	Botucatu/ São Paulo State	65	13348-3	Capão Bonito	65
14780		65	13371-2	Miguelópolis	81
14781	Jaú/ São Paulo State	65	13371-3	Miguelópolis	81
14782	Botucatu/ São Paulo State	65	13371-4	Miguelópolis	81
14783		65	13371-5		81
14784	Campinas/ São Paulo State	65	13371-6	Miguelópolis	81
14785	Botucatu/ São Paulo State	81	13373-1	Barretos	81
14786	Itatiba/ São Paulo State	81	13373-3	Barretos	83
14787	Curitibanos/ Santa Catarina State	*	13373-4	Barretos	83
14788	Capão Bonito/ São Paulo State	65	14345-1	Mococa	81
14789	Mococa/ São Paulo State	65	14345-2	Mococa	81
14790	Campos Novos/ Santa Catarina State	65	14345-3	Mococa	81
14791		*	14345-4		81
14827	Avaré/ São Paulo State	*	14345-5	Mococa	81
14828		*			

Races classified according to inoculation response in differential cultivars proposed by Pastor-Corrales (1991). *Non-pathogenic isolates.

According to Ishikawa et al. (2012), this wide variability can also occur from fusions by conidial anastomosis tubes (CAT), which provides the source of the pathogen variants. The diversity of the pathogen variability by CAT was also studied in the *Glomerella cingulata* (Stonem.) Spauld. et Schrenk f. sp. *phaseoli*, the sexual form of *C. lindemuthianum,* where the authors reported their ability to achieve sexual recombination as well the ability to form CAT. Moreover, they report the possibility of CAT formation between *C. lindemuthianum* and *G. cingulata*, increasing the pathogen variability (Barcelos, Pinto, Vaillancourt, & Souza, 2014). The genetic diversity of *C. lindemuthianum* was also reported by molecular markers using IRAP (Inter-retrotransposon Amplified Polymorphism) and Remap (retrotransposon Microsatellite), indicating high variability between groups of the pathogen and effectiveness in perform genetic diversity analysis (Santos et al., 2012; Gonzaga, Costa, Santos, Araujo, & Queiroz, 2014).

Thus, periodically mapping common bean production regions is important for assisting breeding programs to develop resistant cultivars. Although genetic resistance is considered the most economical control strategy also with less environmental impact, the broken resistance of these cultivars is probably due to the wide use in successive crops, leading to a selection pressure and giving rise to pathogen race variants. Therefore, breeding programs have invested in new strategies to prolong the cultivars' resistance, such as pyramiding multiple genes in a single plant variety.

In agreement with the literature, the present study also showed high physiological variability among isolates of race 65. The isolate 14790 was considered the most aggressive among the 13 tested, pathogenic to eight cultivars. Isolates 14835 and 14834 were pathogenic to six; isolates 14789, 14788, 14652, and 14782 were pathogenic to five; and the other isolates, 14779, 14783, 14781, 14784, 1467, and 14780, were pathogenic in four cultivars or fewer (Figure 1). The interaction between *P. vulgaris* and *C. lindemuthianum* was studied by Campa, Rodríguez-Suárez, Giraldez, and Ferreira (2014), and the authors reported that this interaction is specific and complex, conditioned by the pathogenic variation and by the genotype. They identified by mapping the RIL's population 'Cornell49242 x Xana' two resistance genes for race 65, which exhibited a dominance action mode unlike the other races, which exhibited different action modes in both parents and might be eventually addictive or dominant.

All cultivars tested in this study exhibited divergent reactions to the isolates. The cultivar Ouro Vermelho was resistant to all of the isolates tested, this cultivar may be used in crosses to introduce the resistance gene in new genotypes and the cultivar BRS Estilo was susceptible to 10 isolates, reacting similarly to the Pérola cultivar, which is used as a control for susceptibility to the pathogen. This result was also reported by Ishikawa et al. (2011), who observed that the BRS Estilo cultivar was susceptible to 9 of the 12 isolates evaluated, and Ouro Vermelho was resistant to all of the isolates.

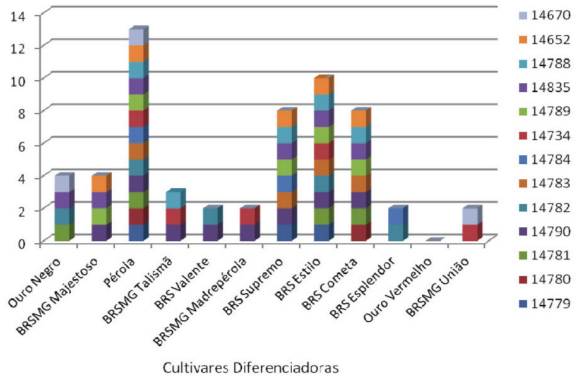

Figure 1. Number of *Colletotrichum lindemuthianum* isolates compatible with each differential cultivar of race 65 of the pathogen.

According to Faria et al. (2004) and Abreu et al. (2011) the cultivars BRSMG Talismã and BRSMG Madrepérola were released as resistant to race 65; however, in this study, the BRS Talisman cultivar showed a susceptible reaction to three isolates of race 65 and BRSMG Madrepérola was susceptible to two, showing a high variability for this race and proving the resistance was broken in these cultivars. According to Melotto and Kelly (2000) and Chiorato, Carbonell, Moura, Ito, and Colombo (2006) a strategy for reducing the susceptibility of cultivars to the wide variability of the pathogen is the insertion of both resistant gene pools (Andean and Mesoamerican) therefore, this combination could provide the most wide and durable resistance. This behavior likely occurs because the Andean cultivars tend to be more resistant to Mesoamerican races, which have high variability and aggressiveness.

The results of this study showed the importance of incorporating new differential cultivars to identify the pathogenic variability of *C. lindemuthianum* isolates in different regions of Brazil. This

evaluation is highly relevant for the identification of the most aggressive isolates of each race of the pathogen, assisting Genetic Breeding Programs in incorporating new sources of resistance in commercial common bean cultivars as a tool to minimize the resistance breaking of widely used cultivars in the production system by the pathogen variability.

Conclusion

Ten races of *C. lindemuthianum* were identified in the state of São Paulo, but only race 65 was identified in the state of Santa Catarina.

Races 65 and 81 were notable for their high frequency among the isolates classified, and races 38 and 351 were identified in Brazil for the first time.

The isolates of race 65 showed wide physiological variability, and it is necessary to incorporate new differential cultivars to identify the pathogen variability levels.

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