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Characterization of *Colletotrichum lindemuthianum* Isolates from the State of Minas Gerais, Brazil

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Abstract

Anthracnose disease of common bean (*Phaseolus vulgaris*), caused by *Colletotrichum lindemuthianum*, is responsible for extensive yield losses worldwide. This pathogen is known to vary greatly in its pathogenicity. Control strategies include chemical control and, mainly, the development of resistant cultivars, taking into account the population structure of *C. lindemuthianum*. The objective of this study was to investigate the pathogenic and genetic diversity and population structure among *C. lindemuthianum* isolates collected in Minas Gerais state, Brazil. When these isolates were inoculated on 12 differential cultivars, a total of 10 races were identified within a series of 48 isolates collected in Minas Gerais, Brazil. Races 65, 81 and 73 were the most frequent races and occurred in most of the regions. This study also detected race 337, which had not been reported previously in the literature. Random amplified polymorphic DNA (RAPD) analysis performed on the same 48 isolates revealed great genetic diversity, clustering the series into five groups at a maximum similarity value of 89.6%. There was no clear relationship between the loci sampled by RAPD markers and the pathogenic characterization. Analysis of molecular variance showed that 96.06% of the variability was contained within regions and 3.94% among regions, indicating a high exchange of genetic material among the regions of the State. Most of the variability was detected within races (75.24%). The pathogenicity and RAPD assays corroborated the broad genetic diversity of the pathogen and the results have been useful in breeding for resistance to anthracnose.

Introduction

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner is one of the most important diseases in common bean and can be devastating when climatic conditions favour the pathogen. Losses due to disease can reach to 100% (Chaves, 1980; Rava et al., 1993, 1994; González et al., 1998) and control strategies include, mainly, use of clean seed, chemical control and the development of resistant cultivars. In Brazil, most bean production is by medium to small or subsistence farmers who do not apply fungicides due to high costs. As a result, breeding for disease resistance is the most effective, safest, practical and economically accessible strategy to control anthracnose disease.

Despite this, the major limitation for developing durable resistance in common bean cultivars is the magnitude of variability in *C. lindemuthianum* reported worldwide (Rava et al., 1993, 1994; Balardin, 1997; Balardin and Kelly, 1998; González et al., 1998; Mahuku and Riascos, 2004; Talamini et al., 2004). High levels of pathogenic (90 races) diversity have been identified among 200 isolates, revealing *C. lindemuthianum* to be a highly diverse pathogen (Mahuku and Riascos, 2004). Furthermore, evolving races of *C. lindemuthianum* continue to overcome specific resistance genes in *Phaseolus vulgaris* (Kelly, 1995; Balardin and Kelly, 1998). Knowledge of the predominant races of *C. lindemuthianum* will allow breeders to adopt strategies to improve durable resistance, such as pyramiding resistance genes in *P. vulgaris*. Therefore, understanding pathogenic variability is a fundamental part of a breeding programme (Alzate-Marin and Sartorato, 2004).

The genetic diversity of *C. lindemuthianum* has been investigated using random amplified polymorphic DNA (RAPD) marker analysis (Balardin et al., 1997; Sicard et al., 1997; González et al., 1998; Mesquita et al., 1998; Thomazella et al., 2002; Mahuku and Riascos, 2004), amplified fragment length polymorphism (González et al., 1998) and random amplified microsatellites

(RAMs) (Mahuku and Riascos, 2004). These studies have revealed that *C. lindemuthianum* is a highly variable pathogen that maintains high levels of genetic diversity. Mahuku and Riascos (2004) showed that the diversity exhibited by *C. lindemuthianum* does not appear to cluster according to common bean gene pools, while the high diversity found in the Mesoamerican region seems to indicate that *C. lindemuthianum* originated and was disseminated from this region.

Combining virulence studies with molecular analysis will lead to a better understanding of the variability present in *C. lindemuthianum*, information that is crucial for designing anthracnose management strategies, deploying resistance genes and developing resistant commercial bean cultivars (Mahuku and Riascos, 2004).

The objective of this study was therefore to analyse the pathogenic and genetic diversity of *C. lindemuthianum* isolates collected in Minas Gerais state, so as to generate data to be used in breeding programmes for resistance to common bean anthracnose.

Materials and Methods

C. lindemuthianum race identification

Fungal isolates. Isolates of *C. lindemuthianum* obtained from naturally infected common bean cultivars were used in this study. Forty-eight isolates of the bean anthracnose fungus were collected from four regions of Minas Gerais state, Brazil: Buriti, Coromandel, Monte Carmelo and Patos de Minas (Alto Paranaíba Region); Januária and Unaí (North Minas Region); Lambari, Lavras and Luminárias (South Minas Region) and Viçosa (Forest Zone Region), as shown in Table 1. Small pieces of infected plant tissue were surface-sterilized and placed on Petri dishes containing M3 culture medium, and each isolate was purified by single spore isolation technique and multiplied in bean pods embedded in M3 medium.

Identification of races. The set of 12 differential cultivars proposed by International Center for Tropical Agriculture (CIAT, 1990), plus the susceptible cultivar Carioca, was inoculated with each *C. lindemunthianum* isolate (Table 1). Sixteen seeds were sown for each differential cultivar.

To obtain high sporulation levels, sterile young green bean pods were inoculated with a suspension of each isolate of *C. lindemuthianum* and were incubated at 22 ± 2°C for 8–10 days in darkness. A conidial suspension was obtained by filtering the homogenate through two layers of cheesecloth to remove mycelial fragments. The spore concentration was estimated using a hemocytometer and adjusted to a final concentration of 1.2×10^6 conidia/ml using sterile distilled water. Ten-day-old bean seedlings, with fully expanded primary leaves, were sprayed with the conidial suspension until runoff on the stem and both surfaces of the unifoliolate leaves using a De Vilbiss air compressor. Inoculated plants were incubated in a humidity chamber (20 ± 2°C, 95% relative humidity) for 48 h with a 12-h photoperiod.

Table 1
Races and provenances (county and regions of Minas Gerais state, Brazil) of the *Colletotrichum lindemuthianum* isolates investigated

Isolate	Races	County[a]	Region[b]	Isolate	Race	County	Region
1	65	BUR	AP	25	81	LV	SM
2	65	BUR	AP	26	81	LV	SM
3	69	BUR	AP	27	81	LV	SM
4	81	BUR	AP	28	81	LV	SM
5	65	CO	AP	29	81	LV	SM
6	65	MC	AP	30	89	LV	SM
7	65	MC	AP	31	89	LV	SM
8	81	PM	AP	32	65	LU	SM
9	81	PM	AP	33	65	VI	ZM
10	87	PM	AP	34	65	VI	ZM
11	65	JA	NM	35	65	VI	ZM
12	81	UN	NM	36	65	VI	ZM
13	65	LM	SM	37	65	VI	ZM
14	65	LM	SM	38	65	VI	ZM
15	8	LV	SM	39	81	VI	ZM
16	64	LV	SM	40	81	VI	ZM
17	65	LV	SM	41	81	VI	ZM
18	65	LV	SM	42	83	VI	ZM
19	65	LV	SM	43	87	VI	ZM
20	73	LV	SM	44	87	VI	ZM
21	73	LV	SM	45	337	VI	ZM
22[c]	73	LV	SM	46	337	VI	ZM
23[c]	73	LV	SM	47	337	VI	ZM
24[c]	73	LV	SM	48	337	VI	ZM

[a]County: BU, Buriti; CO, Coromandel; MC, Monte Carmelo; PM, Patos de Minas; JA, Januária; UN, Unaí; LM, Lambari; LV, Lavras; LU, Luminárias; VI, Viçosa.
[b]Regions: AP, Alto Paranaíba Region; NM, North Minas Region; SM, South Minas Region; ZM, Forest Zone Region.
[c]Isolates of sexual stage (*Glomerella cingulata* f. sp. *phaseoli*).

Plants were transferred to greenhouse benches. Disease reactions were scored 7–10 days after inoculation based on a 9-point descriptive scale (Schoonhoven and Pastor-Corrales, 1987). On this scale, resistant phenotype scores of 1–3 are assigned to plants with limited or no symptoms and no fungus sporulation (incompatible reaction) whereas plants that were scored 4 or greater are considered susceptible (compatible reaction). Inoculations were repeated at least twice for each isolate. The identified races were assigned a value based on the binary nomenclature system (Habgood, 1970). The differential cultivars were arranged in a fixed order and receive a fixed value. This value was defined by the following equation: 2^{n-1}, where 2 was the number of reaction classes taken into consideration (resistant or susceptible) and n is the order number of the cultivar. An incompatible reaction has a value of zero and nullifies the cultivar value when multiplied by it. The compatible reaction has a value of 1 and gives the same cultivar value when multiplied by it. So, the total sum of the values from the compatible reaction, gives the number that designates the race.

DNA manipulation and analysis

Mycelium from each of the 48 isolates was plated on Petri dishes containing M3 medium (Junqueira et al., 1984) and incubated at 22°C for 12 days. Agar plugs taken from the actively growing margins of the colonies were transferred to Erlenmeyer flasks, each containing

125 ml of M3 liquid culture medium. The flasks were then incubated under constant agitation at 110 rpm at 20°C in the dark for 7 days. The mycelial mass was filtered through cheesecloth, washed with 0.05 M EDTA, and kept at −20°C for DNA extraction.

The DNA was extracted according to the (modified) methodology developed by Raeder and Broda (1985).

The RAPD reactions were carried out with the primers OP A13, OP AQ11, OP AS19, OP BA03, OP BA06, OP BA08, OP BB01, OP BB03, OP BB05, OP BB08, OP BB12, OP BB13, OP BB15 and OP BB19 (Operon Technologies, Alameda, CA, USA). Amplification reactions were performed in thermocycler model Eppendorf MasterCycler Gradient 5331 (Eppendorf, Hamburg, Germany) in a 14 μl final volume containing 4 μl water, 35 ng of genomic DNA and 50 μM of each dNTP, 0.4 μM oligonucleotide primer, 50 mM Tris-HCl, pH 8.0, 2.0 mM $MgCl_2$, 20 mM KCl and 0.6 units *Taq* DNA polymerase enzyme.

Amplification was programmed for one initial denaturation cycle (94°C for 2 min), followed by 38 cycles of 2 min at 94°C, 15 s at 37°C and 1 min at 72°C and a final extension step of 2 min. Amplification products were separated by electrophoresis in 1.0% agarose gel and visualized under UV light (Fotodyne Ultraviolet Trans-illuminator; Fotodyne Inc., New Berlin, WI, USA) before being photographed with a Kodak EDA – 290 camera (Eastman Kodak Company, Rochester, NY, USA).

The DNA bands obtained for each individual were scored based on their presence (1) or absence (0). Only polymorphic bands were considered. The genetic similarities between individuals were estimated by using the Nei and Li coefficient (Nei and Li, 1979). The similarity matrix generated was then used to produce a dendrogram by the unweighted pair group method with arithmetic (UPGMA) using the NTSYS-PC 2.1 program.

The errors associated with each similarity were estimated according to the following expression modified from Skroch et al. (1992).

$$\text{Estimated standard error:}(s_{\text{sg}}) = \sqrt{\text{sg}_{ij}\frac{1-\text{sg}_{ij}}{n-1}},$$

where n is the sum of a, b and c for each isolate pair.

The genetically different isolates were identified in the dendrogram based on the estimated maximum significant similarity value (sg_m). The sg_m was estimated by a t-test, using the following expression:

$$\text{sg}_m = 1 - \left(t \times \bar{s}_{\text{sg}}\right),$$

wherein t is the tabled value of the Student's t distribution at the 1% level of probability with $n - 2$ degrees of freedom and $\bar{s}_{\text{sg}}$ is the mean error of the comparisons considered in the dendrogram.

Analysis of molecular variance

The analysis of molecular variance (AMOVA) was performed by using the ARLEQUIN 2.000 program (Schneider et al., 2000). The analyses used a hierarchical structure in which each region (except North Minas) and each physiological race (except races 8, 64, 69, 83 and 89) were considered as populations.

The total genetic variance σ_T^2 corresponded to $\sigma_a^2 + \sigma_b^2$.

Variance components were tested using the ϕ_{ST} coefficient, obtained from the expression.

$$\phi_{\text{ST}} = \frac{\sigma_a^2}{\sigma_T^2},$$

where ϕ_{ST} is equivalent to the ratio of variances among categories (regions or races) divided by the total variance.

Results and Discussion

The isolates analysed in this study presented different patterns of virulence when inoculated on the 12 differential cultivars (Table 2). The 48 isolates studies were classified into 10 distinct races. These results confirm that *C. lindemuthianum* is pathogenically highly variable, in agreement with Mahuku and Riascos (2004) who identified high levels of pathogenic (90 races)

Table 2
Reaction of differential cultivars to the isolates of *Colletotrichum lindemuthianum* and identification of races

Races	Differential cultivars[a]												Number of isolates
	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	2^{11}	
8	–[b]	–	–	+[c]	–	–	–	–	–	–	–	–	1
64	–	–	–	–	–	–	+	–	–	–	–	–	1
65	+	–	–	–	–	–	+	–	–	–	–	–	18
69	+	–	+	–	–	–	+	–	–	–	–	–	1
73	+	–	–	+	–	–	+	–	–	–	–	–	5
81	+	–	–	–	+	–	+	–	–	–	–	–	12
83	+	+	–	–	+	–	+	–	–	–	–	–	1
87	+	+	+	–	+	–	+	–	–	–	–	–	3
89	+	–	–	+	+	–	+	–	–	–	–	–	2
337	+	–	–	–	+	–	+	–	+	–	–	–	4
Isol/dif. cv.[d]	46	4	4	8	22	0	47	0	4	0	0	0	48

[a]Michelite (2^0), Michigan Dark Red Kidney – MDRK (2^1), Perry Marrow (2^2), Cornell 49-242 (2^3), Widusa (2^4), Kaboon (2^5), Mexico 222 (2^6), PI 207262 (2^7), TO (2^8), TU (2^9), AB136 (2^{10}) and G2333 (2^{11}).
[b]Incompatible reaction (−).
[c]Compatible reaction (+).
[d]Isol/dif. cv. – number of isolates that presented compatible reaction with each differential cultivar.

diversity among 200 isolates. Race 65 was the most frequent (18 isolates) and the most widely distributed in Minas Gerais state. Other races that presented high frequencies were 81 and 73, with 12 and 5 isolates, respectively (Table 2). These results reveal the need of breeding programmes to develop cultivars with resistance genes to these races and that the best strategy should involve pyramiding resistance genes. Others studies carried out in Brazil have shown that races 65, 73 and 81 are the most frequent (Rava et al., 1994; Balardin et al., 1997; Carbonell et al., 1999; Somavilla and Prestes, 1999; Sartorato, 2002; Talamini et al., 2002, 2004).

The low frequency of occurrence of other races, however, does not mean that they should be ignored. Attention should be paid to containing the appearance of new virulence alleles capable of 'breaking' the resistance of those alleles widely used in breeding programmes. Therefore, adequate planning should be adopted, observing the regions of occurrence of races and host cultivars, to delay the dissemination or arrival of new races in determined regions, and consequently, prolong the useful life of the cultivars indicated for cropping.

In this study, the sexual form of *C. lindemuthianum* fungus was identified for the first time in natural populations. This important finding could explain part of the wide variability presented by *C. lindemuthianum*, despite the sexual stage being rare in nature (Mahuku and Riascos, 2004). Pathogens with mixed reproduction systems have significant advantages over strictly asexual or strictly sexual pathogens. During the sexual cycle, many new combinations of alleles are produced and mutations will be recombined into many different genetic backgrounds (McDonald and Linde, 2002).

The identification of race 337 is also of great importance as it 'breaks' allele *Co-4* resistance present in the TO differential cultivar (Table 2), which has been used as a source of resistance in several breeding programmes. The occurrence of new races could have arisen by a loss of avirulence factors (Alzate-Marin et al., 1997). Race 337 infects one extra differential cultivar (TO) compared with race 81, which was one of the most frequent in Minas Gerais state in last years (Abreu et al., 1993). It is speculated that mutation within pathogen populations leads to a loss of avirulence factors, producing a compatible reaction with cultivars that were previously resistant (Alzate-Marin et al., 1997). The breeding programmes could use, for example, cultivar G2333 (*Co-4*^{2}, *Co-5* and *Co-7* genes) as a source of resistance in crosses with commercial cultivars which aim to develop bean cultivars with long-lasting resistance to the anthracnose pathogen by resistance genes pyramids.

In order to determine whether the pattern of infection of the differential cultivars by *C. lindemuthianum* isolates is a general pattern observed in other studies, a comparison was made of the infection patterns of all races reported in Minas Gerais state in the last 10 years (Fig. 1b; Rava et al., 1994; Vilarinhos et al., 1995; Alzate-Marin et al., 1997; Mesquita et al., 1998; Sartorato, 2002; Talamini et al., 2002, 2004). These compiled results indicate that the cultivars most frequently found to be susceptible in other studies are Mexico 222, Michelite, MDRK, Perry Marrow, Widusa, Cornell 29242, Widusa and Kaboon. A comparison of Fig. 1a,b shows that the pattern of infection, in general, was similar but in the new data, cultivar TO was infected. This comparison with the data compiled from the literature emphasizes a good sampling of the races, and also shows a low tendency of modification in the pattern of infection in differential cultivars. Similar results have been obtained by González et al. (1998).

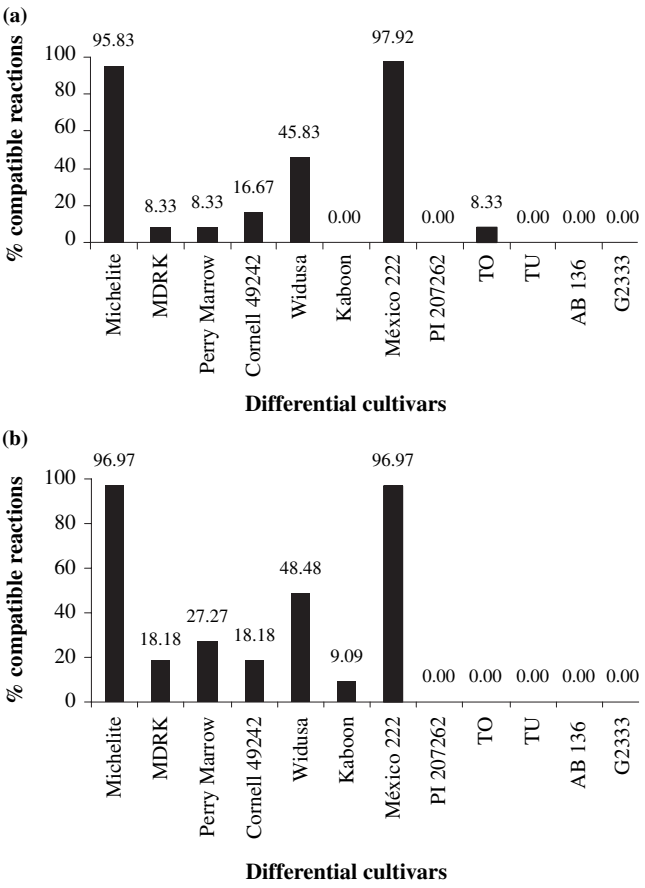

Fig. 1 Percentage of compatible reactions between differential cultivars and evaluated isolates. (a) Present data; (b) literature compiled data in last 10 years

Nine of the 10 distinct races presented compatible reactions with the differential cultivar Mexico 222 (*Co-3*) and eight with the differential cultivar Michelite (Table 2). Talamini et al. (2002) studied isolates derived from different common bean crop regions in Minas Gerais and verified that the differential cultivars Michelite and Mexico 222 were susceptible to 16 of the 17 isolates evaluated. Similar results have also been reported in other surveys (Rava et al., 1994; Balardin, 1997; Carbonell et al., 1999; Talamini et al., 2004).

The differential cultivars Kaboon (*Co-1*^{2}), PI 207262 (*Co-4*^{3} and *Co-9*), TU (*Co-5*), AB136 (*Co-6* and *co-8*)

and G2333 (*Co-4*^{2}, *Co-5* and *Co-7*) were not infected by any races (Fig. 1a), showing that those cultivars are important sources of resistance for a breeding programme to control anthracnose. Carbonell et al. (1999) and Talamini et al. (2002) observed that the resistance of cultivars TO, TU, AB 136 and G2333 was not broken.

Table 3 shows the geographic distribution of the races. Lavras and Viçosa showed the greatest isolate frequency (68.75%). Some races occurred only in one location, such as races 8, 64, 73 and 89 in Lavras, 69 in Buriti, 83 and 337 in Viçosa. However, races such as 65 and 81 were found in eight and five locations, respectively, among the 10 evaluated, showing evidence of wide distribution and adaptation in Minas Gerais state. This wide distribution may have been generated by the free grain trade within the state and especially by the use of susceptible cultivars such as Perola and Carioca.

Some studies have underlined the wide dissemination and adaptation of these two races in Brazil (Rava et al., 1994; Balardin et al., 1997; Carbonell et al., 1999; Talamini et al., 2002, 2004). The occurrence of only one race was reported in some locations, showing that knowledge of the variability of the fungus in each region is an important basis to establish a breeding programme for resistance to anthracnose.

RAPD analysis

A total of 64 polymorphic bands were used to analyse the 48 *C. lindemuthianum* isolates. An average of 4.57 bands was generated per primer. The genetic similarity among the isolates ranged from 0.65 to 0.99 (Fig. 2).

The cut-off line represents the maximum similarity value (s_{gm}) to the right of which, the accessions are considered similar. The s_{gm} at the 1% probability level was 0.896. The dendrogram showed the occurrence of five groups. Group I contained 55% of the isolates from the South Minas Gerais region and 50% of the isolates from the Forest Zone.

Eight of the 10 isolates from the Alto Paranaíba Region were also classified in this group. Group II was formed by two isolates, one from the Forest Zone Region (37 ZM) and the other from the South Minas Region (17 SM). Two isolates from the South Region (13 SM and 19 SM) formed Group III. The four isolates from the Forest Zone were classified as race 337 and formed Group IV. Group V was formed by isolates of the sexual phase (*Glomerella cingulata* f. sp. *phaseoli*) of the *C. lindemuthianum* fungus.

The results do not give evidence of geographical differentiation for *C. lindemuthianum* in Minas Gerais state. This support the conclusions of Fabre et al. (1995), Balardin et al. (1997) and Mahuku and Riascos (2004) who reported that the genetic structure of *C. lindemuthianum* reveals no geographical differentiation. This has important implications in the employment of resistance genes and in directing programmes, which are tasked with developing anthracnose-resistant cultivar. However, some studies showed that *C. lindemuthianum* isolates separated into groups associated with the geographic location from which they were obtained (Alzate-Marin et al., 1997; González et al., 1998; Thomazella et al., 2002). The difficulty of clustering the isolates by region of origin can be explained by the free interchange of seeds infected with *C. lindemuthianum* spores among regions. Furthermore, the breeding programmes are based mainly on the introduction and assessment of a large number of lines of different origins.

Correlation was not observed between the molecular markers and races. Low correlation coefficients between the virulence patterns and the group of RAPD were expected as virulence is a character that has been selected strongly. Other than this, the characters detected by RAPD are neutral, are distributed along the genome and have not been selected. For this reason, it is not out of place that the two types of characters have developed divergent forms and at different rates (Otoya et al., 1995). However, according to Mahuku and Riascos (2004), combining virulence studies with molecular analysis will lead to a better understanding of the variability present in *C. lindemuthianum*, crucial information for designing anthracnose management strategies, deploying resistance genes and developing resistant commercial type bean cultivars.

Table 3
Distribution of *Colletotrichum lindemuthianum* isolates in different regions of Minas Gerais state, Brazil

	Regions[a]									
	AP				NM		SM			ZM
Races	BU	CO	MC	PM	JA	UN	LM	LV	LU	VI
8								1		
64								1		
65	2	1	2		1		2	3	1	6
69	1									
73								5		
81	1			2		1		5		3
83										1
87				1						2
89								2		
337										4
Total isolates	4	1	2	3	1	1	2	17	1	16

[a]Regions: AP, Alto Paranaíba Region; NM, North Minas Region; SM, South Minas Region; ZM, Forest Zone Region. Counties: BU, Buriti; CO, Coromandel; MC, Monte Carmelo; PM, Patos de Minas; JA, Januária; UN, Unaí; LM, Lambari; LV, Lavras; LU, Luminárias; VI, Viçosa.

Analysis of molecular variance

Table 4 shows the summary of the AMOVA obtained from clustering at regional level. Each region, except for North Minas (only two isolates), was considered to be a population. The AMOVA showed that the genetic differentiation among regions is highly significant ($\Phi_{ST} = 0.0394$, $P < 0.016$; 50 175 permutations), with 3.94% and 96.06% of the genetic variability being among regions and within regions (populations), respectively. The free exchange of seeds among regions

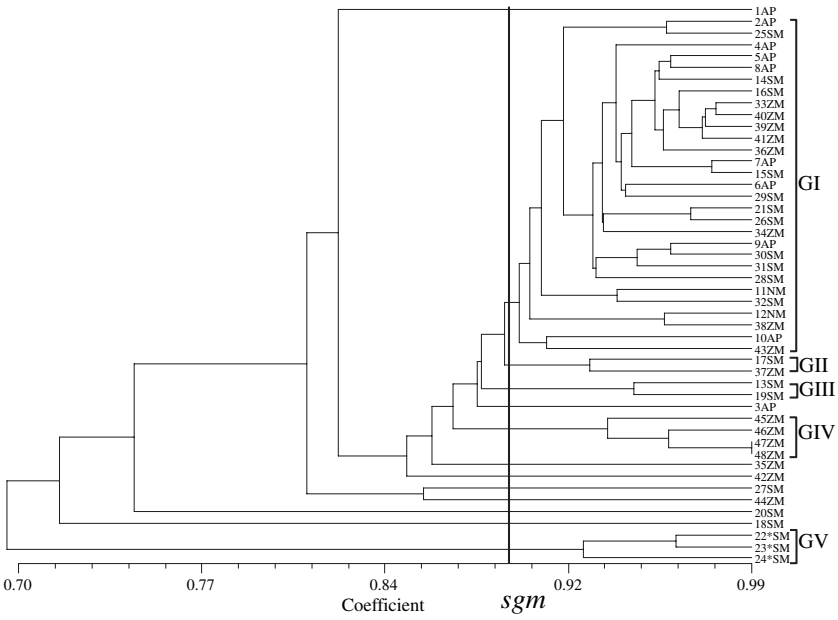

Fig. 2 Dendrogram of genetic similarities among isolates of *Colletotrichum lindemuthianum* from four subregions of Minas Gerais state

Table 4
Summary of analysis of molecular variance of three regions (AP, SM and ZM), from Minas Gerais state of *Colletotrichum lindemuthianum* evaluated for random amplified polymorphic DNA markers

S.V.	df	SS	Variance components	Total (%)	Φ_{ST}	P-value
Among regions	2	23.592	0.3014	3.94	0.0394	0.016
Within regions	43	315.625	7.3401	96.06		
Total	45	339.217	7.6415	100.00		

Table 5
Summary of analysis of molecular variance for seven races of *Colletotrichum lindemuthianum* evaluated for random amplified polymorphic DNA markers

S.V.	df	SS	Variance components	Total (%)	Φ_{ST}	P-value
Among races	4	86.678	2.0727	24.76	0.2476	0.000
Within races	37	232.989	6.2970	75.24		
Total	41	319.667	8.3696	100.00		

may have contributed substantially to the increased variability within regions vs. that among regions.

Different results were reported by Sicard et al. (1997), who studied the genetic diversity among *C. lindemuthianum* isolates originating from three centres of *P. vulgaris* diversity. The authors used AMOVA to show that 58.46% of the diversity was among regions. These results can be explained by the facts that the sampling differed for each country (Mexico, Ecuador and Argentina), there was no exchange of contaminated seeds among the countries and, principally, isolate surveys were carried out in regions where cultivars of different gene pools were cropped (Andean and Mesoamerican). The cultivars used in Brazil are mainly from the Mesoamerican gene pool.

Further clustering at race level was made, where each race (except races 8, 64, 69, 83 and 89) was considered a population, and an AMOVA was performed (Table 5). Again, the greater part of the variability was detected within races (75.24%). It was also observed that the genetic variability among races was highly significant ($\Phi_{ST} = 0.2476$, $P < 0.000$; 50 175 permutations). The high among- and especially within-race variability detected showed the need for good sampling of races and numbers of isolates and demonstrated the consistency of the sampling performed in this study.

Different isolates belong to the same race when they are present in common the same pathogenicity genotype. However, the loci that were sampled by RAPD markers were not necessarily identical for the same race, justifying the high variability found within the populations, because, as was observed, there was no relationship between the molecular markers and the races characterized by the differential cultivars. Most of the among-race variability resulted, for example, from what occurred with race 65 isolates in Groups II and III (Fig. 2). The races in these groups may have originated from different races by mutation and convergent directional selection. Conversely, the great number of different races within the same group, given their high degree of kinship, may have originated from mutations in the same race.

Thus, it was shown that there was large within-race variability, which validated studies emphasizing the great potential of this fungus to generate variability, and the need to assess the mechanisms involved in obtaining this genetic variability (González et al., 1998; Camargo, 2004).

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References

Abreu AFB, Ramalho MAP, Menu HMR. Identificação de raças de *Colletotrichum lindemuthianum* do Sul e Alto Paranaíba de Minas Gerais. 4th Reunião Nacional de Pesquisa de Feijão, Londrina, Brazil, IAPAR, 1993, p. 45.

Alzate-Marin AL, Sartorato A. (2004) Analysis of the pathogenic variability of *Colletotrichum lindemuthianum* in Brazil. *Annu Rep Bean Improv Coop* **47**:241–242.

Alzate-Marin AL, Baía GS, Faleiro FG et al. (1997) Análise da diversidade genética de raças de *C. lindemuthianum* que ocorrem em algumas regiões do Brasil por marcadores RAPD. *Fitopatol Bras* **22**:85–88.

Balardin RS. (1997) Identificação de raças fisiológicas de *C. lindemuthianum* no Rio Grande do Sul – Brasil. *Fitopatol Bras* **22**:50–53.

Balardin RS, Kelly JD. (1998) Interaction between *Colletotrichum lindemuthianum* races and gene pool diversity in *Phaseolus vulgaris*. *J Am Soc Hortic Sci* **123**:1038–1047.

Balardin RS, Jarosz AM, Kelly JD. (1997) Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central, and North America. *Phytopathology* **87**:1184–1191.

Camargo OA Jr. *Identificação de recombinantes de Glomerella cingulata f. sp. phaseoli por meio de marcadores RAPD*. Master's thesis, Lavras, Brazil, Universidade Federal de Lavras, 2004, p. 60.

Carbonell SAM, Ito MF, Pompeu AS, Francisco FG, Ravagnani S, Almeida ALL. (1999) Raças fisiológicas de *Colletotrichum lindemuthianum* e reação de cultivares e linhagens de feijoeiro no Estado de São Paulo. *Fitopatol Bras* **24**:60–65.

Chaves G. La antracnosis. In: Schuartz HF, Galvez GE (eds), *Problemas de producion de fríjol: enfermidades, insectos, limitaciones edáficas y climáticos de Phaseolus vulgaris*, Cali, Colombia, CIAT, 1980, pp. 37–53.

CIAT. *Informe Anual 1988: programa de frijol*. Cali, Colombia, CIAT, 1990, pp. 128–129 (CIAT, Documento de Trabajo, 72).

Fabre JV, Julien J, Parisot D, Dron M. (1995) Analysis of diverse isolates of *Colletotrichum lindemuthianum* infecting common bean using molecular markers. *Mycol Res* **99**:429–435.

González M, Rodríguez R, Zavala ME et al. (1998) Characterization of Mexican isolates of *Colletotrichum lindemuthianum* by using differential cultivars and molecular markers. *Phytopathology* **88**:292–299.

Habgood RM. (1970) Designation of physiological races of plant pathogens. *Nature* **227**:1268–1269.

Junqueira NTV, Chaves GM, Zambolin L, Romeiro RS, Gasparotto R. (1984) Isolamento, cultivo e esporulação de *Mycrocyclus ulei*, agente etiológico do mal-das-folhas da seringueira. *Rev Ceres* **31**:322–331.

Kelly JD. (1995) Use of random amplified polymorphic DNA markers in breeding for major genes resistance to plant pathogens. *HortScience* **30**:461–465.

Mahuku GS, Riascos JJ. (2004) Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. *Eur J Plant Pathol* **110**:253–263.

McDonald BA, Linde C. (2002) The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica* **124**:163–180.

Mesquita AGG, Paula T Jr, de Barros EG. (1998) Identification of races of *Colletotrichum lindemuthianum* with the aid of PCR-based molecular markers. *Plant Dis* **82**:1084–1087.

Nei M, Li WH. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA* **76**:5269–5273.

Otoya MM, Restrepo S, Pastor-Corrales MA. (1995) Amplificación al azar del ADN polimórfico para evaluar la diversidad genética de *Colletotrichum lindemuthianum* en Colômbia. *Fitopatol Colomb* **19**:7–14.

Raeder U, Broda P. (1985) Rapid preparation of DNA from filamentous fungi. *Appl Microbiol* **1**:17–20.

Rava CA, Molina J, Kauffman M, Briones I. (1993) Determinación de razas fisiológicas de *Colletotrichum lindemuthianum* en Nicarágua. *Fitopatol Bras* **18**:388–391.

Rava CA, Purchio AF, Sartorato A. (1994) Caracterização de patótipos de *Colletotrichum lindemuthianum* que ocorrem em algumas regiões produtoras de feijoeiro comum. *Fitopatol Bras* **19**:167–172.

Sartorato A. Determinação da variabilidade patogênica do fungo *Colletotrichum lindemuthianum* (Sacc.) Scrib. 7th Congresso Nacional de Pesquisa de Feijão, Viçosa, Brazil, Universidade Federal de Viçosa, 2002, pp. 114–116.

Schneider S, Roessli D, Excoffier L. *Arlequin ver. 2. 000: A Software for Population Genetics Data Analysis*. Switzerland, University of Geneva, Genetics and Biometry Laboratory, 2000.

Schoonhoven AV, Pastor-Corrales MA. *Standard System for the Evaluation of Bean Germoplasma*. Cali, Colombia, Centro Internacional de Agricultura Tropical, 1987.

Sicard D, Michalakis Y, Dron M, Neema C. (1997) Genetic diversity and pathogenic variation of *Colletotrichum lindemuthianum* in the three centers of diversity of its host, *Phaseolus vulgaris*. *Phytopathology* **87**:807–813.

Skroch P, Tivang J, Nienhuis J. Analysis of genetic relationships using RAPD marker data. Applications of RAPD Technology to Plant Breeding, 1992, Minneapolis, Proceedings, Minneapolis, Crop Science Society of America, 1992, pp. 26–30.

Somavilla L, Prestes AM. (1999) Identificação de patótipos de *Colletotrichum lindemuthianum* de algumas regiões produtoras de feijão do Rio Grande do Sul. *Fitopatol Bras* **24**:416–442.

Talamini V, Souza EA, Pozza EA, Fernandes FR, Ishikawa FH. Identificação de raças de *Colletotrichum lindemuthianum* de regiões produtoras de feijão-comum em Minas Gerais. 7th Congresso Nacional de Pesquisa de Feijão, Viçosa, Brazil, Universidade Federal de Viçosa, 2002, pp. 187–189.

Talamini V, Souza EA, Pozza EA et al. (2004) Identificação de raças patogênicas de *Colletotrichum lindemuthianum* a partir de isolados provenientes de regiões produtoras de feijoeiro comum. *Summa Phytopathol* **30**:371–375.

Thomazella C, Gonçalves-Vidigal MC, Vidigal Filho OS, Barelli MAA, Silvério L. Divergência genética entre e dentro de raças de *Colletotrichum lindemuthianum*, determinada por marcadores RAPD. 7th Congresso Nacional de Pesquisa de Feijão, Viçosa, Brazil, Universidade Federal de Viçosa, 2002, pp. 35–37.

Vilarinhos AD, Paula Jr TJ, de Barros EG, Moreira MA. (1995) Characterization of races of *Colletotrichum lindemuthianum* by the random amplified polymorphic DNA technique. *Fitopatol Bras* **20**:194–198.