Genetic relatedness of Brazilian *Colletotrichum truncatum* isolates assessed by vegetative compatibility groups and RAPD analysis

JULIANE R SANT'ANNA¹*, CLÁUDIA T MIYAMOTO¹*, LÚCIA J ROSADA¹,
CLAUDINÉIA C S FRANCO¹, EDILSON N KANESHIMA²
and MARIALBA A A CASTRO-PRADO¹**

Universidade Estadual de Maringá.

¹ Departamento de Biologia Celular e Genética.

² Departamento de Análises Clínicas. Avenida Colombo 5790. Maringá, PR 87020-900, Brasil.

ABSTRACT

The genetic variation among nine soybean-originating isolates of *Colletotrichum truncatum* from different Brazilian states was studied. Nitrate non-utilizing (*nit*) mutants were obtained with potassium chlorate and used to characterize vegetative compatibility reactions, heterokaryosis and RAPD profile. Based on pairings of *nit* mutants from the different isolates, five vegetative complementation groups (VCG) were identified, and barriers to the formation of heterokaryons were observed among isolates derived from the same geographic area. No complementation was observed among any of the *nit* mutants recovered from the isolate A, which was designed heterokaryon-self-incompatible. Based on RAPD analysis, a polymorphism was detected among the wild isolate C and their *nit1* and *NitM* mutants. RAPD amplification, with five different primers, also showed polymorphic profiles among Brazilian *C. truncatum* isolates. Dendrogram analysis resulted in a similarity degree ranging between 0.331 and 0.882 among isolates and identified three RAPD groups. Despite the lack of a correlation between the RAPD analysis and the vegetative compatibility grouping, results demonstrated the potential of VCG analysis to differentiate *C. truncatum* isolates genotypically similar when compared by RAPD.

Key terms: genetic variability, heterokaryosis, parasexual recombination, RAPD.

INTRODUCTION

Anthrachnose is one of the most destructive fungal diseases of soybean (*Glycine max*) causing considerable losses in soybean-growing regions worldwide, especially when warm and humid conditions exist, favoring pathogen development. The disease reduces stand, seed quality and yields. In certain Brazilian regions anthrachnose is one of the most important phytosanitary factors causing total soybean yield losses. Anthrachnose affects all aboveground parts of the vegetable soybean and the typical symptoms, characterized by brown and irregularly shaped spots, appear

during early reproductive stages on stems, pods and petioles. Although seedling infections may occur, anthrachnose may also attack more mature plants during the latter part of the growing season (Almeida et al., 2005; Chen et al., 2006).

The most common pathogen associated with soybean anthrachnose is the filamentous fungus *Colletotrichum truncatum* (Schwein.) Andrus & W. D. Moore, (teleomorphic *Glomerella truncata* C.L. Armstr. & Banniza), belonging to the class of Pyrenomycetes. This class includes many species of *Colletotrichum* causing anthrachnose in a wide range of crops, such as lentils (*Lens culinaris*), fababeans (*Vicia*

* These authors contributed equally to this work.

** Corresponding Author: M. A. A. Castro-Prado, Phone: (+55) 44 3261 4679, Fax: (+55) 44 3261 4893, E-mail: maacprado@uem.br.

faba) and peas (*Pisum sativum*), and even in ornamental plant species (Freeman et al., 2000; Curry and Baird, 2004).

Isolates of *C. truncatum* vary considerably in colony characteristics, size of fruiting structures and pathogenicity. Additionally, *C. truncatum* from different hosts often exhibit different host preferences. Previous studies have demonstrated that *C. truncatum* isolates from lentils and peas infected and sporuled on both hosts, but were unable to infect soybean (Weidemann et al., 1988; Anderson et al., 2000). On the other hand, isolates from hemp sesbania (*Sesbania exaltata*) were specific to that host (Boyette, 1991).

RAPD markers and rDNA sequence data have been utilized to assess the genetic variability of *C. truncatum* isolates infecting lentils, soybeans, *Xanthium* sp. and peanuts. Results have shown the genetic relatedness of *C. truncatum* isolates from the same host species and discriminated among isolates from lentils and other host species (Ford et al., 2004). The high degree of genetic variability of fifty-eight *C. truncatum* isolates from urdbean, horsegram, soybean, ricebean, cowpea and mungbean, collected from the northwestern Himalayas, was also demonstrated by RAPD profiles generated by random primers (Sharma, 2007).

Processes of gene mutation, heterokaryosis and mitotic recombination have been proposed to explain the high pathogenic variability of several *Colletotrichum* species (Casela and Frederiksen, 1994; Chacko et al., 1994; Souza-Paccola et al., 2003). The parasexual process has in fact been considered as a possible mechanism for genetic diversity among asexual and phytopathogenic fungi (Caten, 1981; Zeigler et al., 1997). Heterokaryosis, the association of two genetically different nuclei in a common cytoplasm, and asexual gene exchange, however, are limited by vegetative compatibility loci named *het* (for heterokaryon incompatibility) or *vic* (for vegetative incompatibility), so that only compatible strains, genetically similar to *vic* or *het* genes, may produce stable heterokaryons among themselves. Such

compatible strains are classified as members of the same vegetative compatibility group (VCG) (Leslie, 1993; Busso et al., 2007).

The aims of the current research were to estimate the genetic variability among soybean-originating *C. truncatum* isolates from different Brazilian states, and to assess the diversity of vegetative compatibility reactions among them for a subsequent analysis of the potential for asexual gene exchange in *C. truncatum* isolates.

MATERIAL AND METHODS

Fungal isolates and media

Nine soybean isolates of *C. truncatum* were employed in this study. Isolates A to D were kindly donated by Dr. William Mário de Carvalho Nunes (Departamento de Agronomia, Universidade Estadual de Maringá, Paraná, Brazil), while isolates E to I were obtained from Embrapa Soja, Londrina, Paraná, Brazil (Table I). Culture stocks were maintained at 5°C in amber flasks with Basal Medium. Complete Medium (CM) and Basal Medium (BM) have previously been described by Castro-Prado et al. (2007).

TABLE I

Origin of Brazilian *C. truncatum* isolates.

Isolate	Strain	Area of origin	State
A	52192	Londrina	Paraná
B	0205	Londrina	Paraná
C	33104	Londrina	Paraná
D	28. I	Londrina	Paraná
E	8	Nova Ponte	Minas Gerais
F	10.3.2	Taquarituba	São Paulo
G	19	Guairá	São Paulo
H	17	Bom Jesus	Goiás
I	28.1.1	Rondonópolis	Mato Grosso

Isolation of nit mutants

Mycelium plugs, 5mm in diameter, were removed from the periphery of *C. truncatum* colonies cultivated in CM and transferred to Petri plates with BM + NaNO₃ (0.2%) +

KClO₃ (3.6 %). Plates were incubated at 22°C for 14 days. Mutants that produced poor growth colonies in the presence of potassium chlorate, or rather, without dense aerial mycelium, were classified as unable to use nitrate as a nitrogen source (*nit*). In order to determine the phenotypes of *nit* mutants, they were transferred to Petri plates with BM, supplemented with different nitrogen sources: sodium nitrate (0.2%), potassium nitrite (0.085%), hypoxanthine (0.01%) and ammonium tartrate (0.092%). Plates were incubated at 22°C for 10 days, and *nit* mutants were characterized into two distinct phenotypic classes: *nitI* (nitrate non-utilizing), and *NitM* (hypoxanthine and nitrate non-utilizing) (Klittich and Leslie, 1988).

Hyphal anastomoses

Complementary *nit* mutants from vegetative compatible isolates were paired in all possible combinations to determine their capacity for anastomosis. Mycelium plugs of *nit* mutants were placed 0.5 cm from each other on sterilized microscope slides covered with a thin layer of BM + NaNO₃. Slides were then placed in Petri plates containing the same medium. The plates were incubated at 22° C for 5 days. The mycelium plugs were then removed from the slides; the newly grown hyphae were stained with cotton blue in lactophenol, coverslipped and examined under a light microscope (Fig. 1A).

Figure 1: Pairing of *nit* mutants of Brazilian *C. truncatum* isolates. (A) Hyphae anastomosis (arrow) between *nit* mutants H.4 (*nitI*) and G.13 (*NitM*) from VCG-5. (B) Pairing between *nit* mutants H.4 (*nitI*) and G.13 (*NitM*) separated by a dialysis membrane. Heterokaryons (H) did not occur beneath the dialysis membrane (arrow), which impairs direct contact between the strains' mycelia. (C) Heterokaryotic colony, formed between H.4 and G.13 *nit* mutants, shows mitotic segregation of *nit* paternal mutants (arrows). (D) Heterokaryons (H) formed between *NitM* mutants of isolate G: G.13 (*NitMI*), G.49 (*NitMII*), G.51 (*NitMII*), and G.13 (*NitMI*). Heterokaryons did not occur between the two *NitMII* mutants, G.49 and G.51.

Obtaining heterokaryons

Mycelium plugs (5mm) of *nit* mutant originating from the same and different wild isolates were paired equidistantly apart (approximately 1.0 cm) in Petri plates containing BM + NaNO₃. Plates were incubated at 22°C for 12 to 30 days and then examined for heterokaryotic growth, in the contact area of the paired colonies, and for vegetative complementation group (VCG).

Genetic complementation tests among NitM mutants

NitM mutants were assessed by their ability to complement among themselves by pairing each *NitM* mutant equidistantly apart (approximately 1 cm) from each other in BM + NaNO₃. Plates were incubated at 22°C for 12 to 30 days. *NitM* mutants that did not form heterokaryotic growth in the contact area of the mutant colonies were considered to belong to the same genetic complementation group (GCG).

Cross-feeding test

Two agar blocks cut from the growing edge colonies of two complementary *nit* mutants were placed on opposite sides, approximately 5 cm apart, on Petri plates containing BM + NaNO₃. A permeable cellulose dialysis membrane, which restricts physical contact between hyphae, was placed between the two colonies at the interaction zone. Plates were incubated at 22°C for 15 days and then examined for heterokaryotic growth (Fig. 1B).

Isolation of parasexual segregants

Heterokaryotic mycelium plugs (5 mm) were transferred to plates containing BM + NaNO₃. Two methods were used to detect parasexual segregants (or sectors): (1) isolation of sectors showing *nit* phenotype, directly from the heterokaryotic colonies (Fig. 1C), and (2) suspensions of conidia obtained from heterokaryons colonies growing on BM+ NaNO₃. Aliquots of these suspensions were grown in BM + NaNO₃ to

recover parasexual segregants. Mycelium plugs (5mm) of parasexual segregants were later transferred to BM supplemented with different nitrogen sources for phenotypic characterization.

Genomic DNA extraction

Pure cultures of *C. truncatum* wild isolates and *nit* mutants were grown in CM for 5 days at 25°C. Mycelia were harvested by filtration (Whatman grade 1 filter paper) and ground to a fine powder in liquid nitrogen for DNA extraction, as described by Loudon et al. (1993).

RAPD assay

RAPD reactions were performed using five primers (Operon Technologies, USA): OPA-11 (CAATCGCCGT); OPA-18 (AGGTGACCGT), OPC-08 (TGGACCGG TG); OPF-05 (CCGAATTCCC); and OPW-06 (AGGCCCGCTG) to assess the genetic diversity existing in soybean-originating *C. truncatum* isolates and to detect genetic changes in *nit* mutants derived from *C. truncatum* wild isolates. RAPD amplifications were carried out in a total volume of 25 µL containing 10 mM Tris-HCl, 50 mM KCl, 3 mM MgCl₂, 0.2 mM of each deoxy-nucleotide triphosphate, 10 µM of a single primer, 1.5U Taq DNA polymerase (Invitrogen), and 15 ng of genomic DNA. Temperature cycling conditions were: initial denaturation at 94°C for 5 min, 34 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min, and final extension at 72°C for 5 min. All amplified DNA products were resolved by electrophoresis on polyacrilamide gel (4.5%) in TBE 1X (45mM Tris Borate and 1 mM EDTA) buffer, silver stained as described by Oliveira et al. (1997) and photographed. All experiments were repeated at least twice.

RAPD product scoring and data analysis

DNA bands obtained for each *C. truncatum* wild isolate were scored manually and analyzed as polymorphic and monomorphic bands. Data were compiled as a binary 0/1

matrix by presence (1) or absence (0) of a band at a particular position. The genetic similarities among individuals were estimated by using Jaccard coefficients. Dendrograms were produced by cluster analysis using the unweighted pair-grouped method by arithmetic average (UPGMA).

RESULTS

Isolation of nit mutants and VCG characterization

Nitrate non-utilizing mutants from the wild isolates A to I were obtained in BM + NaNO₃ + KClO₃. Mutants were morphologically characterized by the production of thin expansive colonies on BM containing only nitrate as a nitrogen source. Mutants could be divided in two phenotypic classes that probably represent mutations in the nitrate reductase structural locus (*nitI*) and in loci codifying the cofactor containing molybdenum necessary for nitrate reductase activity (*NitM*). For each of the nine *C. truncatum* wild isolates, up to 30 distinctive *nit* mutants were recovered, with *nitI* mutants as the predominant phenotypic class (Table II).

NitM and *nitI* mutants from the same isolate were paired on BM + NaNO₃ to determine whether the wild isolates were

vegetatively self-compatible. Eight of the nine isolates proved to be self-compatible due to the formation of dense heterokaryons. However, no intra- or inter-isolate complementation occurred for the isolate A when its complementary *nit* mutants were paired with one another or with distinct *nit* mutants of other isolates. Thus, isolate A could not be classified in the vegetative complementation groups.

By pairing *nitI* and *nitM* mutants of isolates B to I in BM + NaNO₃, five distinct vegetative compatibility groups (VCG) were identified: VCG-1 comprised isolates C, F and I; VCG-2 comprised isolate B; VCG-3 formed by isolate D; VCG-4 included isolate E, whereas isolates G and H were classified into VCG-5. Isolates from the same VCG, such as isolates C, F and I from VCG-1, and isolates G and H from VCG-5, carried out anastomosis among themselves (Table II, Fig. 1A).

Pairing of *nit* mutants belonging to the same VCG showed no heterokaryotic growth in BM + NaNO₃ when the paired mutants were separated by a dialysis membrane (Fig. 1B). Results demonstrate the need for physical contact among mutant hyphae (anastomosis) for heterokaryon formation. Furthermore, heterokaryotic colonies growing on plates containing BM + NaNO₃ gave rise spontaneously to auxotrophic sectors (parasexual segregants)

TABLE II

Frequency of *nit* mutants, vegetative compatibility groups (VCG) and genetic complementation groups (GCG) of Brazilian *C. truncatum* isolates A to I.

Isolates	<i>nit</i> mutants (%)		VCG	GCG
	<i>nitI</i>	<i>nitM</i>		
A ¹	86	14	nd ²	nd ²
B	94	06	2	B-I to B-III
C	87	13	1	C-I to C-III
D	87	13	3	D-I to D-V
E	23	77	4	E-I, E-II
F	69	31	1	F-I to F-III
G	93	07	5	G-I, G-II
H	85	15	5	H-I to H-III
I	50	50	1	I-I, I-II

¹ self-incompatible; ²not determined.

that exhibited the parental phenotypes of the crossed mutants (Fig. 1C). The relative proportions of parental types recovered from heterokaryons, however, varied from heterokaryons to heterokaryons (Table III).

Genetic complementation groups (GCG) among NitM mutants

NitM mutants from the same isolate were paired in BM + NaNO₃ to determine how many loci control the production of the molybdenum-containing cofactor in the Brazilian *C. truncatum* isolates. The inability of different *NitM* mutants to form heterokaryon among themselves classifies these mutants as member of the same genetic complementation group (GCG) (Fig. 1D). On the other hand, the heterokaryon formation between paired *NitM* mutants indicates intergenic complementation and classifies these mutants as members of distinct GCGs. For instance, mutants *NitM*-I from isolate B formed heterokaryons with two classes of *NitM* mutants, *NitM*-II and *NitM*-III, but not with other *NitM*-I mutant from the same isolate. Since *NitM*-II and *NitM*-III mutants also formed heterokaryons among themselves, three GCGs (I, II and III)

could be identified in the isolate B (Table IV). Pairings also identified two GCGs (I and II) in isolates E, G, and I; three GCGs (I to III) in isolates C, F and H; and five GCGs (I to V) in isolate D (Table IV). The self-incompatible isolate was not included in these analyses.

RAPD analysis of wild isolates

The five primers generated reproducible amplifications products showing polymorphic profiles among the nine *C. truncatum* isolates. A total of 101 bands were scored, of which 86 (85.15%) were polymorphic. Figure 2 gives an example of the amplifications profiles observed when using random primers. RAPD profiles using primer OPA-11 and OPA-18 were similar for the most of the isolates, except for isolate E (Fig. 2A and B). In fact, a band with approximately 1000 bp (Fig. 2A) has been shown in all isolates, except in isolate E. Contrastingly, bands with approximately 450 bp and 650 bp (Fig. 2B) were shown only by isolate E. Additionally, similar RAPD profiles were observed among the self-incompatible isolate A and the wild C isolate (Fig. 2 A and B).

TABLE III

Recovery of parental phenotypes from heterokaryons formed with Brazilian *C. truncatum nit* mutants

Auxotrophic pairing		Phenotypes of parasexual segregants		
Paternal 1 ¹	Paternal 2 ¹	Paternal 1	Paternal 2	Total ²
C.16 (<i>nitM_{II}</i>) /	C.17 (<i>nitI</i>) ³	74	47	121
C.16 (<i>nitM_{II}</i>) /	C. 37 (<i>nitI</i>) ³	16	14	30
C.16 (<i>nitM_{II}</i>) /	C.10 (<i>nitI</i>) ³	13	24	37
B.15 (<i>nitM_I</i>) /	B.12 (<i>nitI</i>) ³	05	15	20
B.15 (<i>nitM_I</i>) /	B.18 (<i>nitI</i>) ³	03	32	35
B.15 (<i>nitM_I</i>) /	B.26 (<i>nitI</i>) ³	16	51	67
C.16 (<i>nitM_{II}</i>) /	I.37 (<i>nitI</i>) ⁴	27	0	27
I.50 (<i>nitM_{II}</i>) /	C.17 (<i>nitI</i>) ⁴	12	12	24
F.36 (<i>nitM_{III}</i>) /	C.37 (<i>nitI</i>) ⁴	12	30	42
C.16 (<i>nitM_{II}</i>) /	F.19 (<i>nitI</i>) ⁴	69	02	71
G.13 (<i>nitM_I</i>) /	H.4 (<i>nitI</i>) ⁴	14	36	50
H.33 (<i>nitM_{III}</i>) /	G.39 (<i>nitI</i>) ⁴	60	03	63

¹ Strains designated by parent and isolation number, e.g. B.15 is the fifteenth *nit* mutant from isolate B; ²Total number of segregants; ³intrastrain pairing of auxotrophic mutants; ⁴interstrain pairing of auxotrophic mutants.

The genetic similarity among *C. truncatum* isolates ranged from 0.331 (isolate E) to 0.882 (isolates B and F). Cluster analysis identified three different RAPD groups. Group I is composed of isolates A and C, which present a degree of similarity of 0.658. In group II, two sub-groups had been identified, namely, sub-group IIa (isolates B, D and F) and sub-group IIb (isolates G, H and I), with similarity coefficient of 0.821 and 0.781, respectively. Group III is formed only by isolate E, which showed the lowest similarity coefficient (0.331) (Fig. 3).

RAPD analysis of nit mutants

Genetic polymorphism could be identified between the wild isolate C and their auxotrophic mutants C.17 (*nit1*), C.16 (*NitM*), C.60 (*NitM*), and C.62 (*NitM*). In the RAPD analysis obtained with primer OPA-11, a band of 490 bp was amplified from the wild C isolate and from C.17 (*nit1*), C.60 (*NitM*), and C.62 (*NitM*) mutants, but it was not observed in the C.16 *NitM* mutant (Fig. 4A). In the RAPD profiles performed with primer OPA-18, polymorphic bands, with approximately 180

Figure 2: Amplified products of nine wild isolates of *C. truncatum* from different Brazilian states in polyacrilamide gel. RAPD profiles generated by the primer OPA-11 (A) and primer OPA-18 (B). Columns C to D represent isolates from the state of Paraná; column H, from the state of Goiás; columns G and F, from the state of São Paulo; columns I and E, from the state of Mato Grosso and Minas Gerais, respectively; columns M and N, molecular markers and negative control, respectively. Arrows indicate some polymorphic bands.

bp, 220 bp, 300 bp, 350 bp, 400 bp and 1200 bp, were amplified only from the C.17 (*nit1*) mutant. Furthermore, a band with approximately 350 bp was amplified from C.62 (*NitM*) and C.17 (*nit1*) mutants; however, it was not reported in the C.16 and C.60 (*NitM*) mutants (Fig. 4B).

DISCUSSION

Nitrate non-utilizing mutations are auxotrophic markers useful for triggering heterokaryon formation and for testing fungal isolates for vegetative compatibility. *Nit* mutants were relatively easy to recover from *C. truncatum* soybean-isolates, allowing the assessing of most of the isolates for vegetative compatibility. Complementation tests among *nit* mutants showed that vegetative incompatibility barriers were clearly present among soybean-infecting isolates of *C. truncatum*. Three out of the five VCGs, designated VCG-2, VCG-3 and VCG-4, contained only one isolate in each group, whereas VCG-1

and VCG-5 contained three isolates and two isolates, respectively. Only one of the examined isolates, named isolate A, failed to undergo both self and non-self hyphal fusions (Table II). Self-incompatible phenotype has already been reported in *Gibberella fujikuroi*, probably controlled by a single segregating gene (*hsi-1*) (Correl et al., 1989). Since inter-isolate complementation could not be demonstrated using genetically complementary *nit* mutants of isolate A, such self-incompatible isolate was not classified for vegetative compatibility group. In spite of this fact, isolate A showed several RAPD markers in common with the isolate C from VCG-1 (Table II, Figs. 2 and 3).

The absence of heterokaryon formation, when physical contact between the crossed isolates was impaired, indicated that heterokaryon formation was due to direct hyphal fusion and not to cross feeding. Additionally, the recovery of mitotic sectors (or segregants) from the heterokaryon, which exhibited the *nit*

Figure 3: Dendrogram showing genetic similarity between isolates of *C. truncatum* from different Brazilian states based on RAPD profiles.

phenotypes of the paired mutants, indicates that the prototrophic growth of the heterokaryon was not a reversion to prototrophy but a consequence of true heterokaryon formation (Fig. 1B and D, Tables II and III).

Mutants *NitM* from the examined isolates showed intergenic complementation patterns among themselves, suggesting the existence of multiple loci controlling this phenotype in *C. truncatum*. Similar results have also

Figure 4: Polyacrilamide gel showing RAPD profile of *C. truncatum* from wild C isolate and its *nit* mutants. Amplified products were generated by primer OPA-11 (A) and primer OPA-18 (B). Column C, wild C isolate; column C.17, *nitI* mutant; columns C.16 to C.60, *NitM* mutants; columns M and N, molecular markers and negative control, respectively. Arrows indicate some polymorphic bands.

been reported in other *Colletotrichum* species, such as *C. lindemuthianum* and *C. sublineolum* (Castro-Prado et al., 2007; Fávaro et al., 2007). Furthermore, genetic polymorphism was detected by RAPD analysis with primer OPA-18 among *NitM* mutants belonging to the same (C.16 and C.62) and different (C.60) GCGs (Fig. 4B).

RAPD profiles obtained with primers OPA-18 and OPA-11 were able to differentiate the wild C isolate and their auxotrophic C.17 (*nit1*), C.16, C.60 and C.62 (*NitM*) mutants, showing that differences at the DNA level could be detected among phenotypically distinct strains through the molecular method utilized. RAPD markers showed polymorphic profiles among the wild isolates, while a degree of similarity of 0.724 was observed among isolates from RAPD group II (isolates B, D, F, G, H, and I).

Since the vegetative compatibility tests and the RAPD analysis showed that *C. truncatum* isolates could not be clustered according to their geographic origins, the occurrence of free interchange of *C. truncatum* infected seeds among the Brazilian States has been suggested. Isolates C, F and I, from different geographical areas (states of Paraná, São Paulo, and Mato Grosso, respectively), were included into a single VCG (VCG-1) and isolates F and G, from the state of São Paulo, were classified into distinct VCGs, albeit in the same RAPD group II (Table II, Fig. 3). On the other hand, isolates B, D, F, G, H and I, included in the same RAPD group II, could be separated into four different VCGs (Table II, Fig. 3). Results demonstrate the potential of the VCG analysis to differentiate *C. truncatum* isolates genotypically similar when compared by RAPD method. Similar results were obtained in the characterization of *C. acutatum* isolates from Israel and *Fusarium graminearum* isolates from Brazil (Freeman et al., 2000; Busso et al., 2007). Although a correlation between VCG and RAPD analysis has not been observed in present study, isolates G and H, which showed a degree of similarity of 0.816 by RAPD

analysis, were also grouped in the same VCG (Table II, Fig 3).

The genetic variability resulting from the parasexual recombination in fungi was first described by Pontecorvo et al. (1953) in the ascomycete *Aspergillus nidulans*. Subsequently, the term parasexuality was proposed by Pontecorvo (1956) to define processes that result in obtaining recombinant strains without passing through the sexual cycle. The parasexual cycle starts with the formation of the heterokaryon, governed by a rigid genetic system, where vegetatively compatible strains are defined as members of a same VCG (Leslie, 1993; Saupe, 2000). The co-existence of two vegetatively compatible isolates in the same host might lead towards heterokaryon formation. The fusion of two distinct haploid nuclei may then occur inside the heterokaryotic hyphal, originating a diploid nucleus, potentially capable of undergoing mitotic crossing-over, and originating parasexual recombinants, with greater or lesser virulence.

Thus, the parasexual process might be an important factor to increase the genetic variability in *C. truncatum* soybean isolates. Despite the occurrence of parasexual recombination in some *Colletotrichum* species (Chacko et al., 1994; Souza-Paccola et al., 2003), further evidence for asexual gene exchange in *C. truncatum* is required and is dependent on the isolation of stable mitotic recombinants following intrastrain or interstrain pairing of auxotrophic mutants.

REFERENCES

- ALMEIDA AMR, FERREIRA LP, YORINORI JT, SILVA JFV, HENNING AA, GODOY CV, COSTAMILAN LM, MEYER MC. (2005) Doenças da soja (*Glycine max* L.). In: KIMATI H, AMORIM L, BERGAMIN-FILHO A, CAMARGO LEA, REZENDE JAM (eds) Manual de Fitopatologia. 4th ed. São Paulo: Editora Agronômica Ceres, v.2, pp.569-588.
- ANDERSON KL, BUCHWALDT L, SILLERO J, GOSSEN BD (2000) Host range of *Colletotrichum truncatum* from lentil. Can J Plant Pathol 22: 180.
- BOYETTE CD (1991) Host range and virulence of *Colletotrichum truncatum*, a potential mycoherbicide for hemp sesbania (*Sesbania exaltata*). Plant Disease 75: 62-64.

- BUSO C, KANESHIMA EN, FRANCO FA, QUEROL CB AND CASTRO-PRADO MAA (2007) Vegetative compatibility and molecular characterization of *Fusarium graminearum* isolates from the state of Paraná, Brazil. *Rev Ciência Rural* 37: 1813-1816.
- CASELA CR, FREDERIKSEN RA (1994) Pathogenic variability in monoconidial isolates of the sorghum anthracnose fungus *Colletotrichum graminicola* from single lesions and from monococidial cultures. *Fitopatol Bras* 19: 149-153.
- CASTRO-PRADO MAA, QUEROL CB, SANT' ANNA JR, MIYAMOTO CT, FRANCO CCS, MANGOLIN CA, MACHADO MFP (2007) Vegetative compatibility and parasexual segregation in *Colletotrichum lindemuthianum*, a fungal pathogen of the common bean. *Gen Mol Res* 6: 634-642.
- CATEN CE (1981) Parasexual processes in fungi. In: GULL K, OLIVER G (eds) *The Fungal Nucleus*. Cambridge, UK: Cambridge University Press, 191-214.
- CHACKO RJ, WEIDEMANN GJ, TEBEEST DO, CORRELL JC (1994) The use of vegetative compatibility and heterokaryosis to determine potential asexual gene exchange in *Colletotrichum gloeosporioides*. *Biol Control* 4: 382-389.
- CHEN LS, CHU C, LIU CD, CHEN RS, TSAY JG (2006) PCR-based Detection and Differentiation of Anthracnose Pathogens, *Colletotrichum gloeosporioides* and *C. truncatum*, from Vegetable Soybean in Taiwan. *J Phytopathol* 154: 654-662.
- CORRELL JC, KLITTICH CJR, LESLIE JF (1989) Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). *Mycol Res* 93: 21-27.
- CURRY KJ, BAIRD RE (2004) Ascomycota: The filamentous fungi forming Perithecia, Apothecia, and Ascstromata. In: TRIGIANO RN, WINDHAM MT, WINDHAM AS (eds) *Plant pathology, concepts and laboratory exercises*. Florida, CRC press, pp. 127-132.
- FAVARO LCL, ARAÚJO WL, SOUZA-PACCOLA EA, AZEVEDO JL, PACCOLA-MEIRELLES LD (2007) *Colletotrichum sublineolum* genetic instability assessed by mutants resistant to chlorate. *Mycol Res* 111: 93-105.
- FORD R, BANNIZA S, PHOTITA W, TAYLOR PWJ (2004) Morphological and molecular discrimination of *Colletotrichum truncatum* causing anthracnose on lentil in Canada. *Australian Plant Pathology* 33: 559-569.
- FREEMAN S, MINZ D, JURKEVITCH E, MAYMON M, SHABI E (2000) Molecular analyses of *Colletotrichum* species from almond and other fruits. *Phytopathol* 90: 608-614.
- KLITTICH CJR, LESLIE JF (1988) Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics* 118: 417-423.
- LESLIE JF (1993) Fungal Vegetative Compatibility. *Annu Rev Phytopathol* 31: 127-150.
- LOUDON KW, BURNIE JP, COKE AP, MATTHEWS RC (1993). Application of polymerase chain reaction to fingerprint *Aspergillus fumigatus* by random amplification of polymorphic DNA. *J Clin Microbiol* 31: 1117-1121.
- OLIVEIRA RP, MACEDO AM, CHIARI E & PENA SDJ (1997) An alternative approach to evaluating the intraspecific genetic variability of parasites. *Parasitol. Today* 13 (5): 196-200.
- PONTECORVO G (1956) The parasexual cycle in fungi. *Annu Rev Microbiol* 10: 393-400.
- PONTECORVO G, ROPER JA, FORBES E (1953) Genetic recombination without sexual reproduction in *Aspergillus niger*. *J Gen Microbiol* 8: 198-210.
- SAUPE SJ (2000) Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiol Mol Biol Rev* 64: 489-502.
- SHARMA R (2007) Genetic differentiation of host limited forms of *Colletotrichum truncatum* from northwestern Himalayas. *Arch Phytopathol Plant Protect*, DOI: 10. 1080/03235400701543848.
- SOUZA-PACCOLA EA, FÁVARO LCL, CASELA CR, PACCOLA-MEIRELLES LD (2003) Genetic recombination in *Colletotrichum sublineolum*. *J Phytopathol* 151: 329-334.
- WEIDEMANN GJ, TEBEEST TO, CARTWRIGHT RD (1988) Host specificity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* and *C. truncatum* in the Leguminosae. *Phytopathol* 78: 986-990.
- ZEIGLER RS, SCOTT RP, LEUNG H, BORDEOS AA, KUMAR J, NELSON RJ (1997) Evidence of parasexual exchange of DNA in the rice blast fungus challenges its exclusive clonality. *Phytopathol* 87: 284-294.