Brazilian Journal of Microbiology (2003) 34:117-119
ISSN 1517-8382

GENETIC VARIABILITY OF BRAZILIAN *ALTERNARIA* SPP. ISOLATES AS REVEALED BY RAPD ANALYSIS

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Submitted: October 03, 2002; Returned to authors for corrections: January 09, 2003; Approved: February 26, 2003

SHORT COMMUNICATION

ABSTRACT

The genetic variability of 22 isolates of the fungi *Alternaria alternata* and *A. cassiae*, obtained from *Senna obtusifolia*, was studied by RAPD analysis. A total of 491 scorable bands were produced with the use of 28 primers. Cluster analysis based on similarities computed from RAPD markers showed two distinct genetic groups of isolates related to both species. RAPD analysis proved to be an efficient method for detecting genetic variability of *A. cassiae* and *A. alternata* isolates occurring in *S. obtusifolia*, and also for distinguishing *Alternaria* species.

Key words: fungi, *Senna obtusifolia*, bioherbicide, microbial control

Several *Alternaria* species are responsible for diseases in cultivated plants as well in weeds. *Alternaria cassiae* is known as a biological control agent for sicklepod, *Senna obtusifolia,* a highly damaging weed (2,7). In Brazil, sicklepod has been recorded as a serious problem, particularly in soybean crops. The occurrence of another fungus, *Alternaria alternata*, in *S. obtusifolia* was recently detected, increasing the possibilities to develop a bioherbicide to control this weed (4).

Brazilian isolates of *Alternaria* spp. have been characterized based on morphological and isozymes criteria. Cluster analysis obtained from the isozymes data supported the taxonomic identification of species (4).

DNA markers have become a powerful tool to study taxonomy and molecular genetic of a variety of organisms. The random amplified polymorphic DNA (RAPD) allows quick assessment of genetic variability in various taxa, and has been used to study inter and intraspecific variability among isolates of several fungal species used in biological control (3,9,10).

This study was conducted to investigate polymorphism, using RAPD markers, among *A. alternata* and *A. cassiae* isolates obtained from *S. obtusifolia* (Table 1). One *A. solani* isolate obtained from tomato in Brasília, Brazil, was used as an outgroup. Conidia were produced in monoconidial cultures grown on potato dextrose agar (Difco) plates at 28ºC, on the dark, for 10 days. For mycelium production, conidia were inoculated into a semi-synthetic liquid medium (10 g sucrose, 2 g yeast extract, 1 g KH_2PO_4, 0.1 mg $MgSO_4.7H_2O$, 0.44 mg $ZnSO_4.7H_2O$, 0.48 mg $FeCl_3.6H_2O$, 0.36 mg $MnCl_2.H_2O$ in 1 liter of distilled water). Liquid cultures were shaken at 250 rpm at 28ºC, for 7 days. Mycelia were separated from the supernatant by filtration through filter paper (Whatman Nº 1) and stored at -80ºC.

Genomic DNA was obtained from mycelium using a universal rapid salt extraction method (1). PCR reactions were performed in 30- µl volume, with 15 ng of each template, using the PTC-100 programmable thermal controller (MJ Research), and a temperature profile described by Tigano-Milani *et al*. (9). Amplifications were done using the following reaction mix: 1µM of 10-mer primer (Operon Technologies), 200 µM of each dNTP (Pharmacia Biotec), 2 units of Taq DNA Polymerase (Cenbiotec) and 1x of the polymerase´s recommended buffer. Twenty-eight primers were selected for this study: OPB- 03, OPB-04, OPB-05, OPB-06, OPB-07, OPB-08, OPB-10, OPB-11,

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OPB-12, OPB-13, OPB-15, OPB-17, OPB-18, OPB-20, OPC-02, OPC-04, OPC-05, OPC-06, OPC-07, OPC-08, OPC-10, OPC-11, OPC-12, OPC-13, OPC-14, OPC-15, OPC-16 and OPC-19. Amplified products were separated by electrophoresis in 2% agarose gel prepared in 0.5x Tris-borate-EDTA (TBE) buffer, and visualized by staining with ethidium bromide (6) and photographed under UV light.

DNA fingerprints were scored directly from the photographs. Only well resolved products were scored. The presence or absence of each fragment was considered as an independent character. RAPD markers were analyzed using NTSYS-pc V1.8 (5). A similarity matrix was calculated using Jaccard similarity coefficient. Clustering was done using the unweighted mean pair group arithmetic mean method (UPGMA) (8).

Gel electrophoresis of the different sets of RAPD reactions produced 491 scorable bands. On average, 18 bands were produced by the different 28 primers used.

Cluster analysis based on similarities computed from RAPD markers showed two distinct phenetic groups of isolates related to the species *A. alternata* and *A. cassiae* (Fig. 1). These species and the outgroup, *A. solani*, were well separated in this analysis, supporting the taxonomic identification. The group of *A. cassiae* isolates clustered first with the *A. solani* isolate, with approximately 25% similarity, and these two species clustered with the *A. alternata* group with 13% similarity. This analysis also allowed detecting intraspecific variability of *Alternata* spp. isolates obtained from *S. obtusifolia*. In the group of *A. cassiae*, Brazilian isolates clustered at high similarity (>82%), and it was not observed subgroups related to their geographic origin. Therefore, genetic variability of isolates infecting *S. obtusifolia* on the same geographical region was detected. The Brazilian isolates showed also high similarity (>79%) with a isolate from Florida, USA (CG 592). The group of *A. alternata*, including four Brazilian isolates, also showed a high similarity (>79%). In spite being obtained from only one region, the isolates presented genetic variability. This was true even for those isolates collected in the same year (Fig. 1, Table 1). Similar results have been obtained with the analysis of isozymes, using smaller number of isolates (4)

RAPD analysis proved to be an efficient method for detecting genetic variability of *A. cassiae* and *A. alternata* isolates occurring in *S. obtusifolia*, and also for distinguishing *Alternaria* species. However, additional work is needed on *Alternaria* spp. populations pathogenic to *S. obtusifolia,* which includes evaluation of larger sample sizes and isolates from diverse locations.

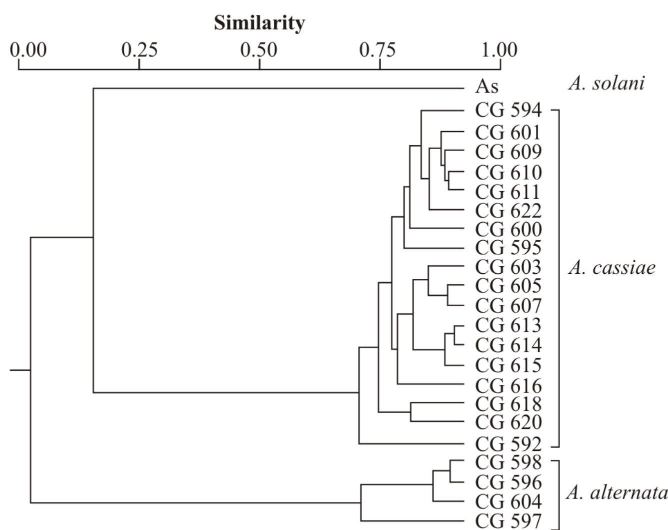

Figure 1. Relationship among *Alternaria* spp. strains as indicated by dendrogram constructed from RAPD analysis data.

Table 1. Source of *Alternaria* spp. isolates obtained from *Senna obtusifolia*.

Species/isolate[a]	Geographical location[b]	Year
A. alternata		
CG598	DF/Brazil	1992
CG604	DF/Brazil	1993
CG596	DF/Brazil	1993
CG597	DF/Brazil	1993
A. cassiae		
CG594	DF/Brazil	1990
CG595	DF/Brazil	1991
CG600	DF/Brazil	1992
CG601	DF/Brazil	1992
CG603	DF/Brazil	1993
CG605	DF/Brazil	1993
CG607	DF/Brazil	1993
CG609	DF/Brazil	1993
CG610	DF/Brazil	1993
CG611	DF/Brazil	1994
CG613	DF/Brazil	1994
CG614	DF/Brazil	1994
CG615	DF/Brazil	1994
CG616	SP/Brazil	1995
CG618	AL/Brazil	1995
CG620	AL/Brazil	1995
CG622	AL/Brazil	1995
CG592	FL/USA	-[c]

[a] Code at Embrapa Genetic Resources and Biotechnology Collection, Brasília, DF, Brazil; [b] Letter abbreviations refer to Brazilian states: AL, Alagoas; DF, Federal District; SP, São Paulo; [c] Unknown.

ACKNOWLEDGMENTS

We thank Bonifácio P. Magalhães, Embrapa Recursos Genéticos e Biotecnologia, for reviewing the manuscript. This work was supported in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

RESUMO

Variabilidade genética de isolados de *Alternaria* spp, revelada através da análise de RAPD

A variabilidade genética entre 22 isolados dos fungos *Alternaria alternata* e *A. Cassiae*, obtidos de *Senna obtusifolia*, foi estudada através da análise de RAPD. No total, 491 bandas foram selecionadas com o uso de 28 primers. O agrupamento baseado em similaridades computadas dos marcadores mostrou dois distintos grupos de isolados relacionados às espécies. A análise de RAPD provou ser um método eficiente para detecção da variabilidade genética entre isolados de *A. alternata* e *A. cassiae* ocorrendo em *S. obtusifolia*, e também para distinguir as espécies de *Alternaria*.

Palavras-chave: Fungos, *Senna obtusifolia*, bioherbicida, controle microbiano

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