ANALYSIS OF MOLECULAR VARIABILITY AMONG ISOLATES OF *ASPERGILLUS FLAVUS* BY PCR-RFLP OF THE ITS REGIONS OF rDNA

*Munusamy Mohankumar, Ayyathurai Vijayasamundeeswari, Muthusamy Karthikeyan, Subramanian Mathiyazhagan, Vaikuntavasan Paranidharan, Rethinasamy Velazhahan**

Department of Plant Pathology, Centre for Plant Protection Studies
Tamil Nadu Agricultural University, Coimbatore- 641 003, Tamil Nadu, India

Received: September 14, 2009
Accepted: September 24, 2010

Abstract: A total of seventeen isolates of *Aspergillus flavus* from maize were collected from different agro-ecological zones of Tamil Nadu, India. The isolates were tested for their ability to produce aflatoxin B_1 (AFB_1) *in vitro* by indirect competitive enzyme-linked immunosorbent assay (ELISA). The amount of AFB_1 produced by the isolates of *A. flavus* ranged from 1.9 to 206.6 ng/ml. Among the various isolates of *A. flavus*, the isolate AFM46 produced the highest amount of AFB_1. DNA was extracted from *A. flavus* isolates and their molecular variability was investigated by using restriction fragment length polymorphism (RFLP) analysis of the PCR-amplified internal transcribed spacer (ITS) regions of ribosomal DNA. PCR amplification with ITS1 and ITS4 primers resulted in the amplification of a product of approximately 600 bp. Digestion of the PCR products with the restriction enzymes EcoRI, HaeIII and TaqI produced fragments of different sizes. Analysis of the genetic coefficient matrix derived from the scores of RFLP profiles showed that minimum and maximum per cent similarities among the tested *A. flavus* strains ranged from 0 to 88%. Cluster analysis using the unweighted pair-group method with arithmetic average (UPGMA) clearly separated the isolates into five groups (group I–V) confirming the genetic diversity among the *A. flavus* isolates from maize.

Key words: aflatoxin B_1, *Aspergillus flavus*, genetic variability, ribosomal DNA internal transcribed spacer (ITS) region, RFLPs

INTRODUCTION

Aflatoxins are a group of highly carcinogenic secondary metabolites mainly produced by *Aspergillus flavus* Link ex. Fries and *A. parasiticus* Spear (Bennett and Christensen 1983). Aflatoxin contamination of various crops including peanut, maize, sorghum and chillies can occur as a result of infection by these fungi. *A. flavus* produces aflatoxins B1 and B2 whereas *A. parasiticus* produces aflatoxins G1 and G2 in addition to B1 and B2. The International Agency for Research on Cancer (IARC) has classified aflatoxin B1, B2, G1 and G2 in group 1 as human carcinogens (IARC 1993). Hence, their quantity in food and feed is closely monitored and regulated in most countries (van Egmond 1995). Variability in aflatoxin production potential of *A. flavus* isolates have been reported (Karthikeyan *et al*. 2009). This is why, the ability to distinguish between the various *Aspergillus* species may have diagnostic value. The analysis of genomic DNA using PCR-based methods has proven to be a fast, sensitive and reliable method for determining genetic relationships among pathogenic microorganisms (Zhang *et al*. 2004; Khoodoo and Jaufeerally-Fakim 2004). Nuclear rDNA, and particularly the internal transcribed spacer (ITS) regions are good targets for the phylogenetic analysis in fungi (Bruns *et al*. 1991) because the ITS regions are often highly variable between isolates of the same species (O'Donnell *et al*. 1998; Salazar *et al*. 1999). In the present study the genetic variability among the isolates of *A. flavus* from maize that vary in their aflatoxin B_1 production potential was analyzed. Restriction fragment length polymorphism (RFLP) analysis of the PCR-amplified ITS regions of ribosomal DNA, was used.

MATERIALS AND METHODS

Survey and collection of samples

Surveys were conducted in different agro-ecological zones of Tamil Nadu, India over a two year period (2005–2006). Pre- and postharvest maize kernel samples were collected from farmers' fields.

Isolation of *Aspergillus* spp.

The fungus, *Aspergillus* sp. was isolated from the collected samples using the potato dextrose agar (PDA) medium under laboratory conditions (Karthikeyan *et al*. 2009). The *Aspergillus* cultures were identified up to the species level using a taxonomic key and species descriptions (Singh *et al*. 1991).

*Corresponding address:
velazhahan@hotmail.com

Aflatoxin B_1 production by various isolates of *A. flavus*

The isolates were analyzed for their aflatoxin B1 production potential in potato dextrose broth. The cultures were grown in 250 ml conical flasks containing 100 ml of potato dextrose broth at room temperature (28±2°C) for 10 days. At the end of the incubation period, the culture filtrate was collected by filtering it through two layers of muslin cloth. The culture filtrate (600 µl) was extracted with chloroform (1 : 1 v/v). The chloroform fraction was evaporated to dryness *in vacuo* and dissolved in 25 µl of 70% methanol and analyzed by ELISA using polyclonal antibodies raised against aflatoxin B1-Bovine serum albumin (AFB_1-BSA) following the method of Reddy *et al*. (2001). Three replicate cultures were analyzed for each isolate.

DNA extraction

A. flavus cultures were grown in 250 ml conical flasks containing 100 ml potato dextrose broth at room temperature (28±2°C) for 5–7 days. Mycelium was harvested by filtration and then freeze-dried. DNA was extracted from ground, freeze-dried mycelium following the method of Liu *et al.* (2000). The extracted DNA was dissolved in TE buffer and stored at –70°C.

DNA amplification

The 5.8S rRNA gene and the two flanking internal transcribed spacers (ITS1 and ITS2) were amplified with primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS 4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White *et al.* 1990). PCR was undertaken in 20 µl volume consisting of 5 mM of each dNTP, 20 pmol each of ITS1 and ITS4 primers, 0.5 U of Taq DNA polymerase and 100 ng of template DNA. Amplifications were done with the following cycling parameters; 94°C for 5 min, 35 cycles of 94°C for 2 min, 53°C for 2 min, 72°C for 2 min, and final extension of 30 min at 72°C. Following amplification, 5 µl of PCR products were digested with the restriction enzymes *Hae*III, *Eco*RI and *Tag*I (Fermentas Inc., Maryland, USA). The digested fragments were separated on 1.2% agarose gel in TAE buffer, stained with ethidium bromide and visualized under UV transillumination. The sizes of the digested products were determined by comparison with standard 1 kb or 100 bp molecular markers (Bangalore Genei Pvt. Ltd., Bangalore, India).

Scoring and statistical analysis

Clearly visible RFLP products were scored from the image of ethidium bromide stained gels. The presence or absence of bands were recorded. For cluster analysis, the NTSYS.PC (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) computer programme was used. The generated pair wise similarity matrix was used to group strains by the unweighted pair-group method with arithmetic average (UPGMA). A dendrogram was derived from the similarity matrix.

RESULTS AND DISCUSSION

In the present study pre- and postharvest maize kernel samples were collected from farmers' fields from different agro-ecological zones of Tamil Nadu, India. *Aspergillus* spp. were isolated from the collected samples using the PDA medium under laboratory conditions. All these isolates were found to be *A. flavus*. A total of seventeen isolates of *A. flavus* were tested for their ability to produce AFB_1 *in vitro* by indirect competitive ELISA. The results indicated that all the isolates produced $AFB_{1,}$ but vary in their level of toxin production. The amount of AFB_1 produced by the toxigenic isolates of *A. flavus* ranged from 1.9 to 206.6 ng/ml (Table 1). Among the various isolates of *A. flavus*, the isolate AFM46 produced the highest amount of AFB_1. These results suggest the existence of variation between isolates of *A. flavus*. The variability in aflatoxin production of *A. flavus* isolates might be due to their genetic makeup (Egel *et al*. 1994). Fungal rDNA has

Table 1. Production of AFB_1 by various isolates of *A. flavus* from maize

No.	Isolate	Origin	AFB_1 production* [ng/ml]
1	AFM13	Poolathur, Coimbatore district	17.1
2	AFM15	Othkalmandabam, Coimbatore district	19.5
3	AFM17	Odaipatty, Dindigul district	1.9
4	AFM19	Kattakamanpatty, Dindigul district	3.9
5	AFM20	Kannivadi, Dindigul district	10.2
6	AFM21	Tharumatthupatty, Dindigul district	58.3
7	AFM23	Nallakaruppanpatty, Theni district	3.9
8	AFM24	Kailasapuram, Theni district	14.9
9	AFM29	Kariyapatty, Madurai district	95.9
10	AFM32	Kalkurichi, Madurai district	20.2
11	AFM40	Ettayapuram,Thoothukudi district	142.8
12	AFM43	Pandalkudi, Thoothukudi district	13.5
13	AFM46	Sinthalakarai, Thoothukudi district	206.6
14	AFM47	Konalai, Viruthunagar district	99.1
15	AFM48	Kapalayam, Viruthunagar district	7.3
16	AFM49	Nedunkur, Viruthunagar district	21.9
17	AFM52	Srugannur, Trichy district	5.2

* each value is the mean of three replicate cultures

been found to contain regions of variability within genera (White *et al*. 1990; Seifert *et al*. 1995; Levy *et al*. 2001). The ITS region of nuclear ribosomal DNA, including ITS1, ITS2 and the intervening 5.8S rRNA gene, has been widely used to study the variability in fungi at the species and sub-species levels (Peterson 1991; Cooke *et al*. 1996; Crawford *et al*. 1996; Cooke and Duncan 1997; Goodwin and Zismann 2001; Appiah *et al*. 2004; Karthikeyan *et al*. 2009). Henry *et al*. (2000) reported sequence variation in several areas in the ITS regions among referenced and clinical isolates of *Aspergillus* species. RFLP analysis of the ITS regions of nuclear rDNA has been used to study the genetic diversity among the isolates of different groups of fungi (Appiah *et al*. 2004). Levy *et al*. (2001) described a PCR-RFLP assay to distinguish *Tilletia walkeri*, a rye grass bunt fungus from *T. indica*, the wheat Karnal bunt fungus. In the present study the *A. flavus* isolates were analyzed by ITS-RFLP to assess the genetic variability among the isolates. PCR amplification of genomic DNA extracted from *A. flavus* isolates with ITS1 and ITS4 primers resulted in the amplification of a product of approximately 600 bp (Fig. 1). The pattern of ITS-RFLP bands

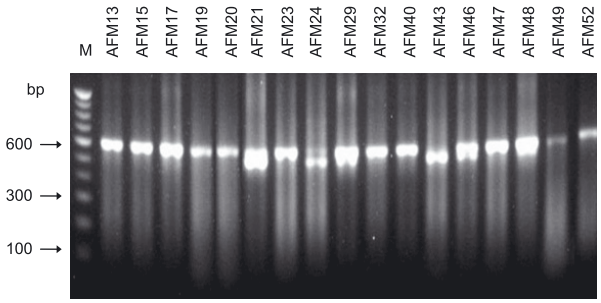

Fig. 1. Agarose gel electrophoresis of internal transcribed spacer (ITS) regions of the ribosomal DNA of *A. flavus.* The 5.8S rRNA gene and the two flanking internal transcribed spacers (ITS1 and ITS2) were amplified by PCR using universal primers ITS1 and ITS4. The PCR amplified products were analyzed by 1.2% agarose gel electrophoresis. Lane; M-100 bp DNA ladder

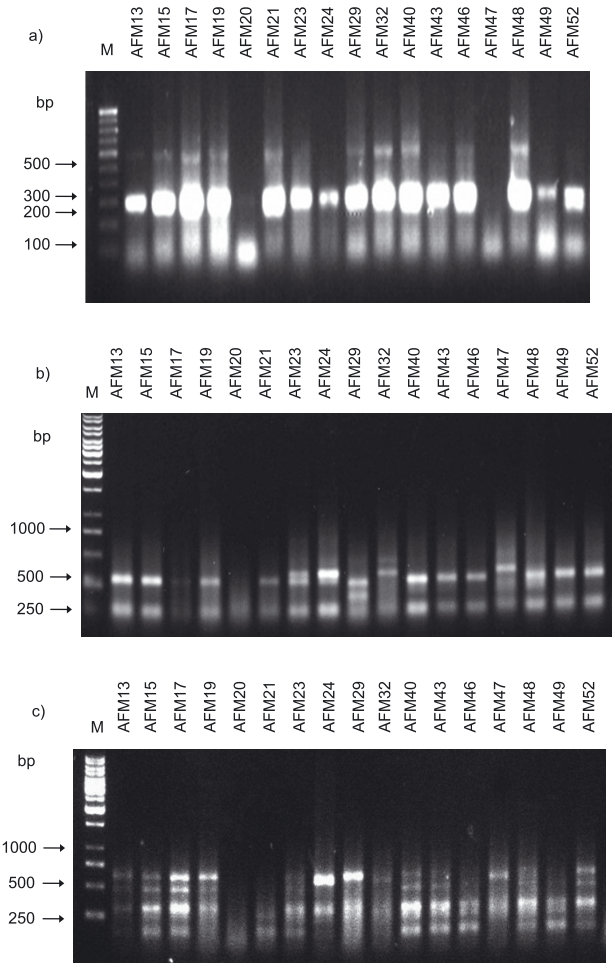

Fig. 2. Restriction patterns of the ITS regions of the ribosomal DNA of *A. flavus* isolates digested with *Eco*RI, *Hae*III and *Tag*I. The 5.8S rRNA gene and the two flanking internal transcribed spacers (ITS1 and ITS2) were amplified by PCR using universal primers ITS1 and ITS4. Following amplification, the PCR products were digested separately with *Eco*RI (a), *Hae*III (b) and *Tag*I (c) and the digested fragments were separated on 1.2 % agarose gel. Lane; M-100 bp ladder

upon digestion of the PCR products with the enzymes *Eco*RI, *Hae*III and *Taq*I indicated genetic variability among the isolates which varied in size of RFLP fragments and number of fragments (Figs. 2a, b, c). Analysis of the genetic coefficient matrix derived from the scores of RFLP profiles showed that minimum and maximum percent similarities among the tested *A. flavus* strains ranged from 0 to 88% respectively (Fig. 3). Cluster analysis using the UPGMA method clearly separated the isolates into five groups (group I–V) (Fig. 4). The isolate AFM20, collected from the Kannivadi, Dindigul district clustered into a separate group (group I). Similarly AFM47, collected from Konalai, Viruthunagar was grouped into group II. Two isolates (AFM29 and AFM32) collected from the Madurai district were clustered together in group III. Group IV contained two isolates (AFM23 and AFM24). Both these isolates were collected from the Theni district. All the remaining strains belonged to group V which consisted of 2 sub-groups. Both highly toxigenic and moderate- or less-toxigenic isolates were found in each group, indicating that no association exists between clustering of *A. flavus* isolates based on ITS-RFLP and the production of aflatoxin. Similar results have been described in other studies with *Aspergillus* spp., showing no correlation between DNA band profiles and toxin production (Bayman and Cotty 1993; Tran-Dinh *et al*. 1999). Lourenco *et al*. (2007) concluded that RAPD was not suitable to discriminate toxigenic strains from non-toxigenic strains of *A. flavus.* Toth *et al*. (2004) reported that no correlation could be observed between clustering of the isolates of *Fusarium culmorum* based on RAPD and mycotoxin-producing abilities or aggressiveness. The total sizes of the restriction fragments in each enzyme digest in the present study exceeded the apparent size of the ITS-PCR product. This difference in size of the fragments could be attributed to the presence of multiple forms of the rDNA-ITS gene cluster in single isolates. This phenomenon is common in fungi and has been reported in *Sclerotium rolfsii* (Harlton *et al*. 1995), *Ascochyta* sp. (Fatehi and Bridge 1998) and *Phytophthora* sp. (Appiah *et al*. 2004).

	AFM13	AFM15	AFM17	AFM19	AFM20	AFM21	AFM23.	AFM24	AFM29	AFM32	AFM40	AFM43	AFM46	AFM47	AFM48	AFM49	AFM52
AFM13	1.00																
AFM15	1.00	1.00															
AFM17	0.77	0.77	1.00														
AFM19	0.77	0.77	0.55	1.00													
AFM20	0.10	0.10	0.00	0.12	1.00												
AFM21	0.66	0.66	0.44	0.62	0.14	1.00											
AFM23	0.60	0.60	0.40	0.40	0.12	0.44	1.00										
AFM24	0.50	0.50	0.30	0.44	0.14	0.33	0.85	1.00									
AFM29	0.46	0.46	0.41	0.41	0.20	0.33	0.30	0.33	1.00								
AFM32	0.45	0.45	0.40	0.40	0.12	0.44	0.40	0.44	0.41	1.00							
AFM40	0.80	0.80	0.60	0.77	0.10	0.66	0.45	0.36	0.35	0.33	1.00						
AFM43	0.80	0.80	0.60	0.60	0.10	0.50	0.60	0.50	0.35	0.33	0.80	1.00					
AFM46	0.66	0.66	0.44	0.44	0.14	0.71	0.62	0.50	0.33	0.44	0.50	0.66	1.00				
AFM47	0.16	0.16	0.09	0.20	0.16	0.10	0.33	0.37	0.15	0.33	0.16	0.16	0.10	1.00			
AFM48	0.63	0.63	0.45	0.45	0.10	0.66	0.45	0.36	0.46	0.45	0.50	0.50	0.66	0.16	1.00		
AFM49	0.55	0.55	0.33	0.33	0.16	0.57	0.71	0.57	0.25	0.33	0.40	0.55	0.83	0.11	0.55	1.00	
AFM52	0.88	0.88	0.66	0.66	0.11	0.55	0.66	0.55	0.38	0.36	0.70	0.88	0.75	0.18	0.54	0.62	1.00

Fig. 3. Genetic similarity coefficient matrix for *A. flavus* isolates based on RFLP of the ITS regions of rDNA indicating the relationship among the isolates

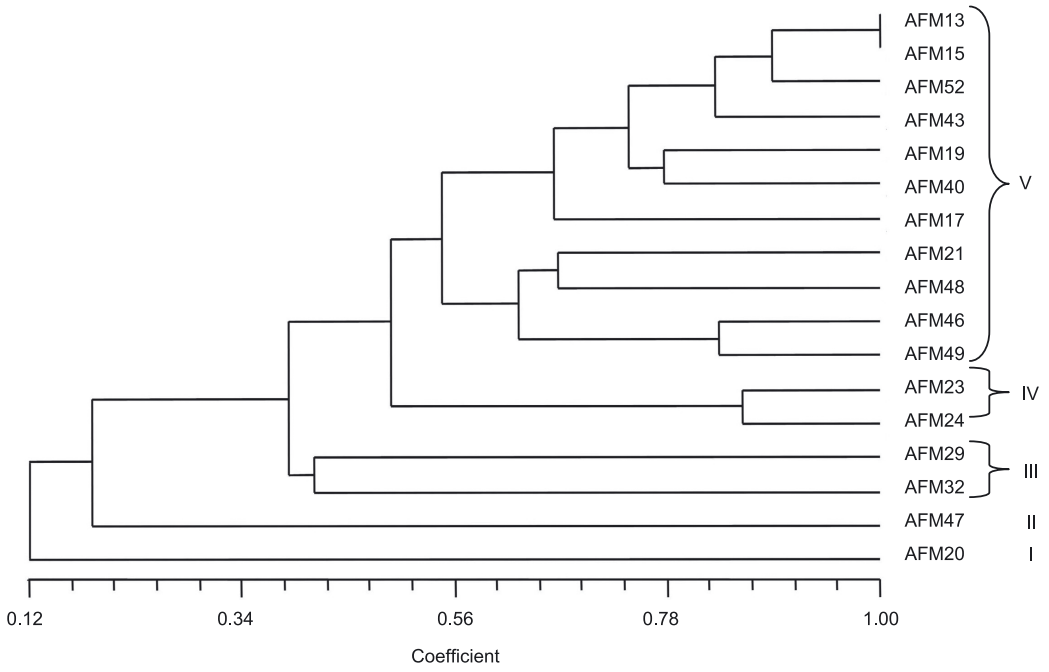

Fig. 4. Unweighted pair group method arithmetic average dendrogram constructed from RFLP data indicating the relationship among the isolates of *A. flavus* from maize

Genetic diversity in fungal population may be of major importance in developing a suitable management strategy. Hence, the genetic variability among the isolates of *A. flavus* should be taken into account when *A. flavus* isolates are used in the resistance breeding programmes for evaluation or to design primers for detection of *A. flavus* in foods and feeds by PCR.

ACKNOWLEDGEMENTS

This study was supported by the Indian Council of Agricultural Research, New Delhi.

REFERENCES

Appiah A.A., Flood J., Archer S.A., Bridge P.D. 2004. Molecular analysis of the major *Phytophthora* species on cocoa. Plant Pathol. 53: 209–219.

Bayman P., Cotty P. 1993. Genetic diversity in *Aspergillus flavus*: association with aflatoxin production and morphology. Can. J. Bot. 71 (1): 23–31.

Bennett J.W., Christensen S.B. 1983. New perspective of aflatoxin biosynthesis. Adv. Appl. Microbiol. 29: 53–92.

Bruns T.D., White T.J., Taylor J.W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. 22: 525–564.

Cooke D.E.L., Duncan J.M. 1997. Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of ribosomal DNA. Mycol. Res. 101 (6): 667–677.

Cooke D.E.L., Kennedy D.M., Guy D.C., Russell J., Unkle S.E., Duncan J.M. 1996. Relatedness of group I species of *Phytophthora* as assessed by random amplified polymorphic DNA (RAPDs) and sequences of ribosomal DNA. Mycol. Res. 100 (3): 297–300.

Crawford A.R., Bassam B.J., Drenth A., MacLean D.J., Irwin J.A.G. 1996. Evolutionary relationships among *Phytophthora* species deduced from rDNA sequence analysis. Mycol. Res. 100 (4): 437–443.

Egel D.S., Cotty P.J., Elias K.S. 1994. Relationships among isolates of *Aspergillus* sect. *Flavai* that vary in aflatoxin production. Phytopathology 84 (9): 906–912.

Fatehi J., Bridge P.D. 1998. Detection of multiple rRNA-ITS regions in isolates of *Ascochyta*. Mycol. Res. 102 (6): 762–766.

Goodwin S.B., Zismann V.L. 2001. Phylogenetic analyses of the ITS region of ribosomal DNA reveal that *Septoria passerinii* from barley is closely related to the wheat pathogen *Mycosphaerella graminicola*. Mycologia 93 (5): 934–946.

Harlton C.E., Levesque C.A., Punja Z.K. 1995. Genetic diversity in *Sclerotium* (*Athelia*) *rolfsii* and related species. Mol. Plant Pathol. 85 (10): 1269–1281.

Henry T., Iwen P.C., Hinrichs S.H. 2000. Identification of *Aspergillus* species using Internal transcribed spacer regions 1 and 2. J. Clin. Microbiol. 38 (4): 1510–1515.

IARC. 1993. IARC–WHO Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 56: "Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Int. Agency for Research on Cancer World Health Organization, 599 pp.

Karthikeyan M., Sandosskumar R., Mathiyazhagan S., Mohankumar M., Valluvaparidasan V., Kumar S., Velazhahan R. 2009. Genetic variability and aflatoxigenic potential of *Aspergillus flavus* isolates from maize. Arch. Phytopathol. Plant Protect. 42 (1): 83–91.

Khoodoo M.H.R., Jaufeerally-Fakim Y. 2004. RAPD-PCR fingerprinting and southern analysis of *Xanthomonas axonopodis* pv. *dieffenbachiae* strains isolated from different aroid hosts and locations. Plant Dis. 88 (9): 980–988.

Levy L., Castlebury L.A, Carris L.M., Meyer R.J., Pimentel G. 2001. Internal transcribed spacer sequence-based phylogeny and polymerase chain reaction-restriction fragment length polymorphism differentiation of *Tilletia walkeri* and *T. indica.* Mycology 91 (10): 935–940.

Liu D., Coloe S., Baird R., Pederson J. 2000. Rapid mini preparation of fungal DNA for PCR. J. Clin. Microbiol. 38, p. 471.

Lourenço A., Durigon E.L., Zanotto P., Madeira J.E.G.C., De Almeida A.P., Correa B. 2007. Genetic diversity of environmental *Aspergillus flavus* strains in the state of São Paulo, Brazil by random amplified polymorphic DNA. Memorias do Instituto Oswaldo Cruz 102: 687–692.

O'Donnell K., Cigelik E., Nirenberg H.I. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90 (3): 465–493.

Peterson S.W. 1991. Phylogenetic analysis of *Fusarium* species using ribosomal RNA sequence comparisons. Phytopathology 81 (9): 1051–1054.

Reddy S.V., Mayi D.K., Reddy M.U., Thirumala Devi K., Reddy D.V. 2001. Aflatoxins B1 in different grades of chillies (*Capsicum annum* L.) in India as determined by indirect competitive ELISA. Food Additiv. Contam. 18 (6): 553–558.

Salazar O., Schneider J.H.M., Julian M.C., Keijer J., Rubio V. 1999. Phylogenetic subgrouping of *Rhizoctonia solani* AG2 isolates based on ribosomal ITS sequences. Mycologia 91 (3): 459–467.

Seifert K.A., Wingfield B.D., Wingfield M.J. 1995. A critique of DNA sequence analysis in the taxonomy of filamentous ascomycetes and ascomycetous anamorphs. Can. J. Bot. 73 (1): 760–767.

Singh K., Frisvad J.C., Thrane U., Mathur S.B. 1991. An Illustrated Manual on Identification of Some Seed-Borne *Aspergilli, Fusaria, Penicillia* and Their Mycotoxins. Danish Government Institute of Seed Pathology for Developing Countries, Denmark, 133 pp.

Toth B., Mesterhazy A., Nicholson P., Teren J., Varga J. 2004. Mycotoxin production and molecular variability of European and American isolates of *Fusarium culmorum*. Eur. J. Plant Pathol. 110 (5–6): 587–599.

Tran-Dinh N., Pitt J.I., Carter D.A. 1999. Molecular genotype analysis of natural toxigenic and nontoxigenic isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. Mycol. Res. 103 (11): 1485–1490.

van Egmond H.P. 1995. Mycotoxins: regulations, quality, assurance and reference materials. Food Additiv. Contam. 12 (3): 321–330.

White T.J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. In: "PCR Protocols: A Guide to Methods and Applications" (M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, eds.). San Diego, CA: Academic Press,

Zhang Z.G., Zhang J.Y., Zheng X.B., Yang Y.W., Ko W.H. 2004. Molecular distinctions between *Phytophthora capsici* and *P. tropicalis* based on ITS sequences of ribosomal DNA. J. Phytopathol. 152 (6): 358–364.

POLISH SUMMARY

ANALIZA MOLEKULARNEJ ZMIENNOŚCI IZOLATÓW *ASPERGILLUS FLAVUS* PRZY WYKORZYSTANIU REGIONÓW rDNA I TECHNIKI PCR-RFLP

Zebrano ogółem siedemnaście izolatów *Aspergillus flavus* z kukurydzy, z różnych stref agro-ekologicznych Tamil Nadu, Indie. Badano zdolność izolatów do wytwarzania aflatoksyny B_1 (AFB_1) *in vitro*, wykorzystując pośredni test ELISA (enzymatyczny test immunosorbcyjny). Ilość wytworzonego AFB_1 przez izolaty *A. flavus* wahała się w granicach od 1,9 do 26,6 mg/ml. Spośród różnych izolatów *A. flavus*, izolat AFM146 wytwarzał największą ilość AFB_1. Ekstrahowano DNA z izolatów *A. flavus* i określano ich molekularną zmienność, wykorzystując analizę poliformizmu długości fragmentów restrykcyjnych (RLFP) regionów rybosomalnego DNA. Amplifikowanie DNA przy wykorzystaniu starterów ITS1 i ITS4, spowodowało amplifikację produktu wynoszącą, w przybliżeniu, 600 bp. Strawienie produktów PCR enzymami restrykcyjnymi EcoRI, HaeIII i TaqI, dało, w wyniku, fragmenty o różnych wartościach genetycznego współczynnika matrix, pochodzącego z ocen. Analiza profilów DNA wykazała, że minimalny i maksymalny procent podobieństwa wśród testowanych ras *A. flavus* wynosił od 0 do 88. Analiza skupień metodą para-grupa przy wykorzystaniu średniej arytmetycznej (UPGMA) wyraźnie dzieliła izolaty na pięć grup (grupy I–IV), potwierdzając genetyczną różnorodność wśród izolatów *A. flavus* z kukurydzy.