UDC 575
https://doi.org/10.2298/GENSR1801143N
Original scientific paper

DIFFERENTIATION BETWEEN *Aspergillus flavus* AND *Aspergillus parasiticus* ISOLATES ORIGINATED FROM WHEAT

Milica NIKOLIC[1], Ana NIKOLIC[1], Marko JAUKOVIC[2], Iva SAVIC[1], Tanja PETROVIC[1], Ferenc BAGI[3], Slavica STANKOVIC[1]

[1]Maize Research Institute, Zemun Polje, Belgrade, Serbia
[2]JV Jugoinspekt Beograd, Belgrade, Serbia
[3]Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia

Nikolic M., A. Nikolic, M. Jaukovic, I. Savic, T. Petrovic, F. Bagi, S. Stankovic (2018): *Differentiation between Aspergillus flavus and Aspergillus parasiticus isolates originated from wheat*.- Genetika, Vol 50, No.1, 143-152.

The species of the genus *Aspergillus*, *A. flavus* and *A. parasiticus*, are the most aflatoxin-producing fungi. All previous studies carried out under the production conditions of Serbia showed no presence of *A. parasiticus* on wheat kernel. On the basis of changes in climatic factors, such as occurrence of high temperatures and prolonged droughts, which favour increased frequency of *Aspergillus* spp., we assumed that this pathogen can also be present in Serbia.

The significance of direct losses as a consequence of wheat kernel infection, as well as potential contamination with aflatoxins, have pointed out to the need to determine the presence of toxigenic potential of *A. flavus* and *A. parasiticus* isolates originating from Serbia. For that purpose, wheat kernel samples were collected in nine locations. According to morphological, toxicological and molecular traits of isolated fungi, the presence of *A. flavus* and *A. parasiticus* was confirmed. This is the first time that *A. parasiticus* was identified on wheat under climatic conditions in Serbia.

This study indicates that these pathogens may be a potential danger in wheat production in the region of Serbia. This danger will be much more certain if global climatic changes continue as they will provide more intensive development of these pathogens.

Key words: aflatoxins, *Aspergillus flavus*, *A. parasiticus*, wheat

INTRODUCTION

Aflatoxins are mycotoxins produced mainly by fungal species *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare, common saprophytes and opportunistic pathogens. These pathogens appear considerably in the tropical and semi-tropical regions on many agricultural

Corresponding author: Milica Nikolic, Maize Research Institute, Zemun Polje, Slobodana Bajića 1, 11185 Belgrade, Serbia,*E-mail: mnikolic@mrizp.rs phone number: +381648406456*

commodities and food matrices. Typical materials, susceptible to aflatoxin contamination, include maize, wheat, rice, groundnuts, pistachios, Brazil nuts, cottonseed, copra and spices (SHEPHARD, 2003). Aflatoxigenic *Aspergillus* species invade susceptible food and feed during pre-harvesting, processing, transportation or storage (DIENER *et al*., 1987).

According to the International Agency for Research on Cancer, aflatoxins are secondary metabolites that are highly toxic and the most carcinogenic substances known (IARC, 2002).

Although *A. flavus* and *A. parasticus* are closely related species, they express some physiological differences, even in the production of mycotoxins. Some of *A. flavus* isolates do not produce aflatoxin, and if they do, they predominantly produce just B aflatoxins. On the other hand, almost all *A. parasticus* isolates produce aflatoxin including B and G toxins (KLICH and PITT, 1988). In general, *A. parasiticus* produces high concentrations of aflatoxin and the majority of strains (over 90%) isolated from natural habitats are capable to synthesise aflatoxins. On the contrary, not more than 40-50% of *A. flavus* isolates have capacity to synthesize toxins (BENNETT and CHRISTNSEN, 1983). The following precise and sensitive methods are used in determination of the aflatoxin concentrations in both, goods and cultures: high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), thin-layer chromatography (TLC), and fluorescence polarisation assay (SEITZ, 1975; TRUCKSESS *et al*., 1994; WHITAKER *et al*., 1996; MARAGOS and THOMPSON, 1999; STROKA and ANKLAM, 2000; NASIR and JOLLEY, 2002; SOBOLEV and DORNER, 2002; ABBAS *et al.*, 2004).

Traditional methods applied in detection and identification of these fungi in foods are based on microscopic and culture techniques and therefore consume time and labour. The development of fast and sensitive methods for the detection and the differentiation of aflatoxigenic species in food are necessary for assessment of the potential health risk of any food. The DNA-based detection methods, such as PCR, are more sensitive, explicit and have been applied in the detection of aflatoxigenic fungi (SHAPIRA *et al*., 1996; FÄRBER *et al*., 1997; SWEENEY *et al*., 2000; CHEN *et al*., 2002). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is a simple technique that uses variations in a DNA sequence. It relates to minor differences in nucleotides that may not be expressed at the protein level. It is broadly applied in the detection and differentiation between mycotoxigenic species (SOMASHEKAR *et al.*, 2004; MARTINEZ-CULEBRAS and RAMON, 2007). Due to the use of this technique *A. flavus* and *A. parasiticus* were successfully distinguished (KHOURY *et al.*, 2011). In order to make the difference between these two species, gene-specific primers were designed to target the intergenic spacer (IGS) for *afl*R and *afl*J genes of the AF biosynthetic cluster.

The identification of *Aspergillus* species is difficult because of great morphological similarity of several species and their ability to cause aspergillosis. The objective of this study was to determine wheat samples containing aflatoxin produced by *A. flavus* and *A. parasiticus*, using morphological, biochemical and molecular criteria. The precise identification of *Aspergillus* spp. is of great importance for prevention and control strategies of fungi and aflatoxins in wheat kernels.

MATERIALS AND METHODS

Plant samples and isolation of Aspergillus strains

The field trial was carried out in nine locations (Zemun Polje, Kraljevo, Šid, Orlovat, Nova Crnja, Bajmok, Srbobran, Bela Crkva and Kikinda). Wheat samples were collected from

field kernels after the harvest in 2015. The isolation of the fungus from wheat kernels was performed by direct plating of wheat kernels after surface sterilisation with bleach/distilled water 1:3. Wheat kernels were placed on potato dextrose agar (PDA) in Petri dishes and incubated at room temperature for 5 days. One hundred kernels per sample (ten per Petri dish x ten replications) were incubated at PDA in order to analyse diversity of *Aspergillus* species.

In the identification and morphological studies, fungi were cultured on PDA, Czapek yeast agar (CYA) and malt extract agar (MEA). *A. flavus* and *A. parasiticus* were identified by using the standard identification keys (RAPER *et al*., 1973; KLICH and PITT, 1988; SAMSON *et al*., 2007; SAMSON *et al*., 2014). The obtained pure cultures of *A. flavus* and *A. parasiticus* have been maintained in the fungal collection of the Maize Research Institute, Zemun Polje, and used to obtain monospore cultures. Monosporial cultures were preserved on slants in the refregerator at 4 °C. A total of 10 isolates of *A. flavus* (Af1 – Zemun Polje, Af2 – Šid, Af3 – Orlovat, Af4 – Nova Crnja, Af5 – Bajmok) and *A. parasiticus* (Ap1 – Zemun Polje, Ap2 – Kraljevo, Ap3 – Srbobran, Ap4 – Bela Crkva, Ap5 – Kikinda), used in our study were isolated from wheat varieties in Serbia. The isolate *A. parasiticus* CBS 100926 was used as positive control.

Morphological identification

Isolates were one point inoculated on the following media: PDA, CYA and MEA in 9-cm plastic Petri dishes using a dense conidial suspension, and incubated in the dark. Macroscopic observations - colony appearance, pigmentation and reverse colouration, as well as microscopic measurements were recorded after a week of growth at 25°C on PDA. The isolates were also grown at 25°C on MEA and CYA. Four Petri dishes were inoculated per medium. Microscopic slides were prepared from 7-day old cultures grown on PDA and MEA. The morphology and diameter of conidia were evaluated.

Sample preparation for the mycotoxin assessment

For the determination of aflatoxins production, isolates were inoculated in the centre of Petri dish using a dense conidial suspension. Isolates were grown on PDA as single colonies in the 9-cm Petri dishes. Cultures were incubated at 28±1°C in the dark for 5 days (ABBAS *et al.*, 2004).

HPLC, extraction and quantitation of aflatoxins

According to ABBAS *et al*. (2004), PDA dishes were scraped into a tube to collect fungal biomass, which was placed in glass vials of known weight and weighed again. Fungal biomass was extracted with a solvent mixture acetonitrile-water (90:10, *v*/*v*) in the ratio of 100:1, *v*/*m*. The vials were shaken for 30 min at high speed using a reciprocal shaker. A 1-mL aliquot of extract was removed and centrifuged at 12 000*g* for 10 minutes. The presence of aflatoxin in the supernatant was assayed by HPLC with fluorescence detection to confirm the presence of aflatoxins (AOAC Official Method 994.08). Data are reported as the mean value of three independent injections.

DNA extraction

A fungal mycelium was obtained from isolates grown on CYA for 2 days at 25°C. DNA extraction from fungal strains was performed according to LUI *et al*. (2000). Total DNA was resuspended in 30 µl of deionised H_2O and stored in the freezer at -20°C until use.

PCR amplification

The intergenic spacer (IGS) for the AF biosynthesis genes *aflJ* and *aflR* was used as a target in order to distinguish *A. flavus* and *A. parasiticus*. The available published regions from the isolates were amplified by a designed primer pair IGS-F/IGS-R (EHRLICH *et al.*, 2003, 2007) that corresponded to a PCR product of 674 bp (KHOURY *et al.*, 2011). The sequences of the primers used were as follows: IGS-F, 5-AAGGAATTCAGGAATTCTCAATTG-3; IGS-R, 5-GTCCACCGGCAAATCGCCGTGCG-3.

Amplification of fungal DNA was performed in a total reaction volume of 50 µl, containing reaction buffer, 1.5 mM $MgCl_2$, 0.8 mM each dNTP (dATP, dCTP, dGTP and dTTP), 1 units *Taq* DNA polymerase, 100 ng template DNA and 0.5 µM of each primer. Amplifications were carried out with the following cycling parameters: initial denaturation at 94˚C for 4 min; 35 cycles of denaturation at 94˚C for 40 s, annealing at 58˚C for 40 s and extension at 72˚C for 1 min; and final extension at 72˚C for 10 min. The PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. PCR amplification of the IGS region yielded a 674 bp band.

Restriction site analysis of PCR products

The analysis of the IGS sequence indicated that the restriction enzyme *Bgl*II cleaved the PCR products into fragments that are useful tools for the detection of *A. flavus* and *A. parasiticus* (KHOURY *et al.*, 2011).

Following amplification, the PCR products were digested with the restriction enzyme *BglII* (Roche, Germany). The reactions were performed in a total volume of 40 µL containing 15 units of enzyme, 4 µL of buffer, 15 µL of the PCR product, and Ultrapure water up to 40 µL. The reaction mixture was incubated at 37°C for 1 h. Then digested fragments were separated by electrophoresis on a 2% w/v agarose gel at 80V for 2.5 h and visualized under UV transillumination. The sizes of the resulting DNA fragments were estimated in comparison with a commercial 100-bp DNA ladder.

RESULTS AND DISCUSSION

Morphological characterisation

Mycelia of the *A. flavus* isolates were yellow-green on PDA, in contrast to mycelia of *A. parasiticus* which were dark green (Figure 1). The reverse was pale yellow. After the 7-day incubation on PDA at 25°C the average colony diameter of *A. flavus* was 7.5 cm, while the corresponding value in the isolates of *A. parasiticus* was 6.5 cm. Isolates of *A. parasiticus* had spherical and rough conidia with thick walls. The *A. flavus* isolates differed by their microscopic traits, as they had thin walls and smooth to finely rough conidia (Figure 2). The average size of conidia of *A. flavus* and *A. parasiticus* was 3.4 µm and 4.4 µm, respectively. Colonies were floccose and thin on MEA and CYA. Mycelia were white, reverse uncoloured on MEA and uncoloured or brown on CYA.

Because of interspecific similarities and intraspecific variability, only morphological differentiation of *Aspergillus* section *Flavi* isolates is not sufficient (RODRIGUES *et al.*, 2009). Therefore, the polyphasic approach is recommended (SAMSON and VARGA, 2009), and it was used in our study.

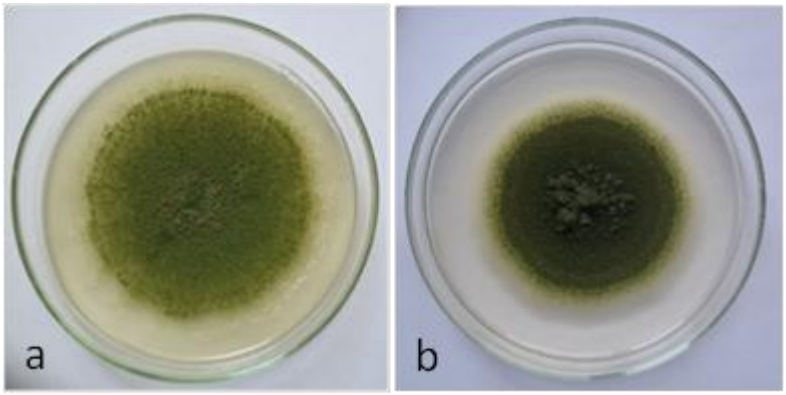

Figure 1. Colony morphology on PDA: a – *A. flavus*, b – *A. parasiticus*

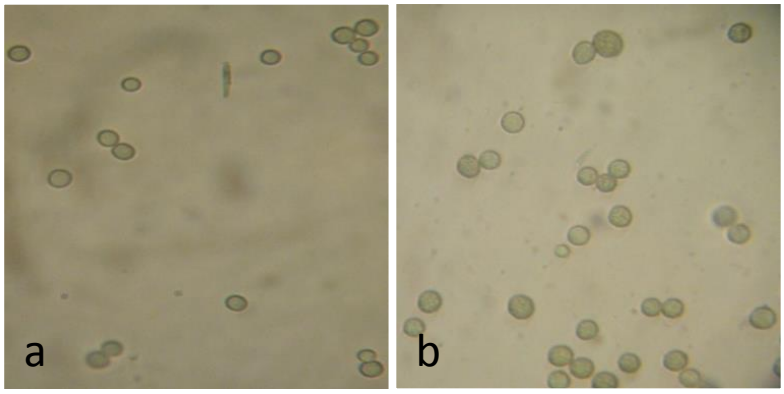

Figure 2. Morphology of conidia on PDA: a – *A. flavus (*globose to ellipsoidal, with smooth to finely roughened walls), b – *A. parasiticus* (globose, distinctly rough-walled)

Toxicological characterisation

Differentiation of *A. flavus* from *A. parasiticus* is of a great importance, because of the differences in metabolite production. *A. parasiticus* synthesises B and G type toxins, whereas *A. flavus* produces only B type toxins (PITT and HOCKING, 2009). Aflatoxin production capability of all *Aspergillus* spp. used in this experiment was analysed with chromatographic technique,

HPLC-FLD. The MRIZP isolates (Af1-Af4) produced aflatoxins B1 and B2, whereas the MRIZP isolate Af5 produced only aflatoxins B1. Moreover, the MRIZP isolates (Ap1-Ap5) produced aflatoxins B1, B2, G1 and G2, except of the isolate Ap2 that did not produce aflatoxin G2 (Table1).

Table 1. Production of aflatoxins by A. flavus and A. parasiticus

	ISOLATES	HPLC			
		B1 (ng/g)	B2 (ng/g)	G1 (ng/g)	G2 (ng/g)
1	Ap1	6.716,417	1.543,802	5.189,430	181.760
2	Ap2	7.361,028	995.407	3.085,115	
3	Ap3	5.168,789	351.969	5.157,556	119.176
4	Ap4	5.708,118	142.807	6.495,071	86.920
5	Ap5	6.431,767	287.250	7.191,624	110.115
6	Af1	6.029,335	302.562	nd	nd
7	Af2	5.698,470	259.575	nd	nd
8	Af3	4.859,150	229.106	nd	nd
9	Af4	5.181,379	318.298	nd	nd
10	Af5	6.460,451	nd	nd	nd

nd - not detected

Genetic characterisation

In order to test the PCR specificity, all fungal strains were amplified using a primer pair IGS-F/IGS-R. Expected amplicons of 674 bp were obtained in all 10 tested isolates of *A. flavus* and *A. parasiticus*. The amplification was not obtained in a negative control (Figure 3). The primers were specific for the *afl*R-*afl*J IGS gene fragment, while the size of amplicons corresponded to the expected size and no additional or non-specific bands were observed.

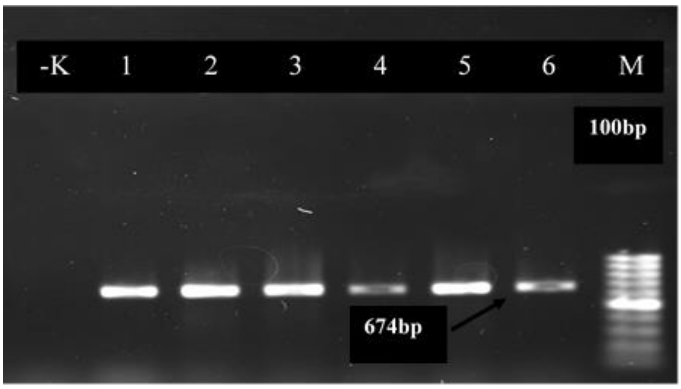

Figure 3. A total of 2% of agarose gel electrophoresis of PCR products with IGSF/IGSR primers. -K – negative control; Lane 1 – *A. flavus* (Af1); Lane 2 – *A. parasiticus* (Ap1), Lane 3 – Ap2, Lane 4 – Ap3, Lane 5 – Ap4; Lane 6 – *A. parasiticus* CBS100926; Lane M – 100 bp DNA marker

The restriction enzyme *BglII* was able to cut the PCR product of *A. flavus* at two sites resulting in three fragments of 362, 210 and 102 bp. On the other hand, there was only one restriction site in the sequence of *A. parasiticus* yielding two fragments of 363 and 311 bp (Figure 4).

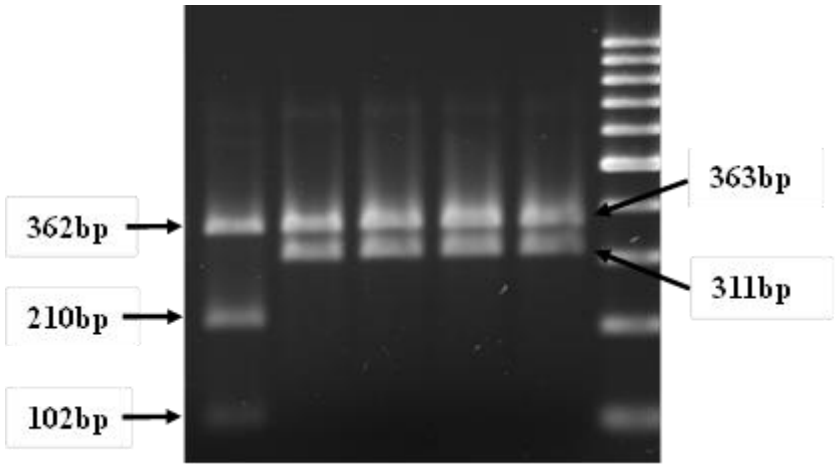

Figure 4. Electrophoretic analysis showing the restriction profiles of the *aflR-aflJ* intergenic spacer PCR product digested with *BglII*. Lane 1 – *A. flavus* (Af1); Lane 2 – *A. parasiticus* (Ap1) Lane 3 – Ap2, Lane 4 – Ap3; Lane 5 – *A. parasiticus* CBS100926; Lane M – 100 bp DNA marker

The distinction between *A. flavus* and *A. parasiticus* is very important since they produce different secondary extrolites. It is well known that *A. flavus* strains produce aflatoxins, cyclopiazonic acid, versicolorin and sterigmatocystin, while *A. parasiticus* specifically produces aflatoxins (WILSON *et al*., 2002). The majority of studies cited in the literature encompass monomeric or multiplex PCR by which aflatoxigenic strains of *A. flavus* and *A. parasiticus* have been detected, but differentiation between them and between non-aflatoxigenic strains has not always been enabled (CRISEO *et al*., 2001). This method is good for the following two reasons: 1) the detection of aflatoxigenic fungi and 2) their differentiation at species level.

CONCLUSIONS

The results obtained have provided an important comparison of *A. flavus* and *A. parasiticus* isolates. The study shows that *A. parasiticus* strains, known as contaminants of maize in fields (STANKOVIĆ *et al*., 2015), were detected in wheat fields. To the best of our knowledge, this is the first report of *A. parasiticus* on wheat kernel not only in Serbia, but in Europe. DOVICICOVÁ *et al*. (2012) identified *A. parasiticus* from wheat kernel in Slovakia, based on morphological examination in conjunction with biochemical analyses, but without rapid and accurate molecular identification.

In order to provide a more holistic approach based on morphological, biochemical and molecular properties of 10 isolates, the causal agents of aspergillosis detected in the wheat field

survey in nine locations in Serbia were identified as *A. flavus* and *A. parasiticus*. Having in mind that these fungi are capable to produce mycotoxins, further studies are necessary to improve strategies for prevention and control of these economically important pathogens.

ACKNOWLEGEMENTS

This research was supported by the project TR31023 and TR31068 funded by the Ministry of Education, Science and Technological Development, Republic of Serbia.

Received, April 24th, 2017
Accepted November 18th, 2017

REFERENCES

ABBAS, H.K., R.M. ZABLOTOWICZ, M.A. WEAVER, B.W. HORN, W. XIE, W.T. SHIER (2004): Comparison of cultural and analytical methods for determination of aflatoxin production by Mississippi Delta *Aspergillus* isolates. Can. J. Microbiol., *50*: 193-199.

AOAC (2000): Official Method 994.08. Aflatoxins in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts.

BENNETT, J.W. and S.B. CHIRSTENSEN (1983): New perspectives on aflatoxin biosynthesis. Adv. Appl. Microbiol., *29*: 53-92.

CHEN, R.S., J.G. TSAY, Y.F. HUANG, R.Y. CHIOU (2002): Polymerase chain reaction mediated characterization of molds belonging to the *Aspergillus flavus* group and detection of *A. parasiticus* in peanut kernels by multiplex polymerase chain reaction. J. Food Protect., *65*: 840-844.

CRISEO, G., A. BAGNAR, G. BISIGNANO (2001): Differentiation of aflatoxin-producing and nonproducing strains of *Aspergillus flavus* group. Lett. Appl. Microbiol., *33*: 291-295.

DIENER, U.L., R.J. COLE, T.H. SANDERS, G.A. PAYNE, L.S. LEE, M.A. KLICH (1987): Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annu. Rev. Phytopathol., *25*: 249-270.

DOVICICOVÁ, M., D. TANCINOVÁ, R. LABUDA, M. SULYOK (2012): *Aspergillus parasiticus* from Wheat grain of Slovak origin and its toxigenic potency. Czech J. Food Sci., *30*(5): 483-487.

EHRLICH, K.C., B.G. MONTALBANO, P.J. COTTY (2003): Sequence comparison of aflR from different *Aspergillus* species provides evidence for variability in regulation of aflatoxin production. Fungal Genet. Biol., *38*: 63-74.

EHRLICH, K.C., K. KOBBEMAN, B.G. MONTALBANO, P.J. COTTY (2007): Aflatoxin-producing *Aspergillus* species from Thailand. Int. J. Food Microbiol., *114*: 153-159.

FÄRBER, P., R. GEISEN, W.H. HOLZAPFEL (1997): Detection of aflatoxigenic fungi in figs by a PCR reaction. Int. J. Food Microbiol., *36*: 215-220.

IARC (2002): Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monogr. Eval. Carcinog. Risks Hum., *82*: 171-300.

KHOURY, A., A. ATONI, T. RIZK, R. LTEIF, M.I. KALLASY, A. MEBRIHI (2011): Differentiation between *Aspergillus flavus* and *Aspergillus parasiticus* from pure culture and aflatoxin and aflatoxin contaminated grapes using PCR-RFLP analysis of *afl*R-*afl*J intergenic spacer. J. Food Sci., *76*(4): 247-253.

KLICH, M.A. and J.I. PITT (1988): Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans. Br. Mycol. Soc., *91*: 99-108.

LUI, D., C. CALOE, R. BIARD and J. PEDERSEN (2000): Rapid mini preparation of fungal DNA for PCR. J. Clin. Microbiol., *38*: 471.

MARAGOS, C.M. and V.S. THOMPSON (1999): Fiber-optic immunosensor for mycotoxins. Nat. Toxins, *7*: 371-376.

MARTINEZ-CULEBRAS, P.V. and D. RAMON (2007): An ITS-RFLP method to identify black *Aspergillus* isolates responsible for OTA contamination in grapes and wine. Int. J. Food Microbiol., *113*: 147-153.

NASIR, M.S. and M.E. JOLLEY (2002): Development of a fluorescence polarization assay for the determination of aflatoxins in grains. J. Agric. Food Chem., *50*: 3116-3121.

PITT, J.I. and A.D. HOCKING (2009): *Aspergillus* and Related Teleomorphs, pp. 275-337. In: Fungi and Food Spoilage, Springer Science+Business Media, New York, USA.

RAPER, K.B., D.I. FENNELL, P.K. AUSTWICK (1973): The Genus *Aspergillus*, Robert E. Krieger Publishing Company, New York.

RODRIGUES, P., A. VENÂNCIO, S. KOZAKIEWICZ, N. LIMA (2009): A polyphasic approach to the identification of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* section Flavi isolated from Portuguese almonds. Int. J. Food Microbiol., *129*: 187-193.

SAMSON R.A., P. NOONIM, M. MEIJER, J. HOUBRAKEN, J.C. FRISVAD, J. VARGA (2007): Diagnostic tools to identify black aspergilli. Stud. Mycol., *59*: 129-145.

SAMSON, R.A. and J. VARGA (2009): What is a species in *Aspergillus*? Med. Mycol., *47*: 13-20.

SAMSON, R.A., C.M. VISAGIE, J. HOUBRAKEN, S.B. HONG, V. HUBKA, C.H.W. KLAASSEN, G. PERRONE, K.A. SEIFERT, A. SUSCA, J.B. TANNEY *et al.* (2014): Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud. Mycol., *78*: 141-173.

SEITZ, L.M. (1975): Comparison of methods of aflatoxin analysis by high-pressure liquid chromatography. J. Chromatogr., *104*: 81-91.

SHAPIRA, R., N. PASTER, O. EYAL, M. MENASHEROV, A. METT, R. SALOMON (1996): Detection of aflatoxigenic molds in grains by PCR. Int. J. Food Microbiol., *62*: 3270-3273.

SHEPHARD, G.S. (2003): Aflatoxin and food safety: Recent African perspectives. J. Toxicol. Toxin Rev., *22*: 267-286.

SOBOLEV, V.S. and J.W. DORNER (2002): Cleanup procedure for determination of aflatoxins in major agricultural commodities by liquid chromatography. Food Chem. Contam., *85*: 642-645.

SOMASHEKAR, D., E.R. RATI, A. CHANDRASHEKAR (2004): PCR-restriction fragment length analysis of *aflR* gene for differentiation and detection of *Aspergillus flavus* and *Aspergillus parasiticus* in maize. Int. J. Food Microbiol., 93: 101-107.

STANKOVIĆ, S., J. LEVIĆ, M. NIKOLIC, V. KRNJAJA, M. JAUKOVIĆ (2015): Prvi nalaz *Aspergillus parasiticus* u proizvodnji kukuruza u Srbiji. Zbornik rezimea XIII Savetovanje o zaštiti bilja, Zlatibor, 23-27.11.2015, 32-33.

STROKA, J. and E. ANKLARN (2000): Development of a simplified densitometer for the determination of aflatoxins by thin-layer chromatography. J. Chromatogr., A *904*: 263-268.

SWEENEY, M.J., P. PÀMIES, A.D.W. DOBSON (2000): The use of reverse transcription polymerase chain reaction (RT-PCR) for monitoring aflatoxin production in *Aspergillus parasiticus* 439. Int. J. Food Microbiol., *56*: 97-103.

TRUCKSESS, M.W., M.E. STACK, S. NESHEIM, R.H. ALBERT, T.R. ROMER (1994): Multifunctional column coupled with liquid chromatography for determination of aflatoxins B1, B2, G1 and G2 in corn, almonds, Brazil nuts, peanuts and pistachio nuts: collaborative study. J. Assoc. Off. Anal. Chem., *77*: 1512-1521.

WHITAKER, T., W. HORWITZ, R. ALBERT, S. NESHEIM (1996): Variability associated with analytical methods used to measure aflatoxin in agricultural commodities. J. Assoc. Off. Anal. Chem., *79*: 476-485.

WILSON, D.M., W. MUBATANHEMA, Z. JURJEVIĆ (2002): Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. Adv. Exp. Med. Biol., *504*: 3-17.

DIFERENCIJACIJA IZMEĐU IZOLATA *Aspergillus flavus* I *Aspergillus parasiticus* POREKLOM S ZRNA PŠENICE

Milica NIKOLIĆ[1], Slavica STANKOVIĆ[1], Ana NIKOLIĆ[1], Marko JAUKOVIĆ[2], Iva SAVIC[1], Tanja PETROVIC[1], Ferenc BAGI[3], Slavica STANKOVIC[1]

[1]Institut za kukuruz "Zemun Polje", Beograd, Srbija
[2]Jugoinspekt Beograd a.d., Beograd, Srbija
[3]Poljoprivedni fakultet, Univerzitet Novi Sad, Novi Sad, Srbija

Izvod

Glavni proizvođači aflatoksina su vrste roda *Aspergillus*, *A. flavus* i *A. parasiticus*. U dosadašnjim istraživanjima, u proizvodnim uslovima Srbije nije zabeleženo prisustvo vrste *A. parasiticus* na zrnu pšenice. Usled promene klimatskih faktora, kao što je pojava visokih temperatura i dugotrajnih suša, koje pogoduju povećanoj učestalosti *Aspergillus* vrsta, pretpostavili smo da pomenuti patogen može biti prisutan i u Srbiji. Značajnost direktnih gubitaka kao posledica zaraza zrna pšenice, kao i potencijalne kontaminacije aflatoksinima, ukazali su na potrebu utvrđivanja prisustva i toksigenog potencijala izolata *A. flavus* i *A. parasiticus* poreklom iz Srbije. U tom cilju, prikupljeni su uzorci zrna pšenice sa šest lokacija. Na osnovu morfoloških, odgajivačkih, toksikoloških i molekularnih svojstava, dobijeni izolati su identifikovani kao *A. flavus* i po prvi put u klimatskim uslovima Srbije - *A. parasiticus.* Ovo istraživanje ukazuje da ovi patogeni mogu biti potencijlna opasnost u proizvodnji pšenice na području Srbije. Ova opasnost bi bila još izvesnija ukoliko bi se globalne klimatske promene nastavile i time omogućile intenzivniji razvoj ovih patogena.

Primljeno 24.IV.2017.
Odobreno 18. XI. 2017.