OCCURRENCE OF POTENTIAL MYCOTOXIN PRODUCING FUNGI ON MAIZE KERNEL IN HUNGARY

Beáta Tóth[1], Orsolya Török[1], Xénia Pálfi[1], Éva Toldi Tóth[1], Ákos Mesterházy[1], János Varga[2]

[1] Cereal Research Nonprofit Ltd., 6726 Szeged, Alsó kikötő sor 9, Hungary
[2] University of Szeged, Faculty of Science and Informatics, Department of Microbiology, 6726 Szeged, Közép fasor 52, Hungary

ABSTRACT

Maize is one of the most important ingredients of animal feed formulations. *Fusarium* species are important pathogens of maize, causing various diseases, and contamination of maize kernel by mycotoxins including trichothecenes, zearalenone and fumonisins. *Aspergillus* and *Penicillium* species and their mycotoxins including aflatoxins, ochratoxins, fumonisins and patulin are also frequently encountered on cereal products. We investigated the occurrence of these species and their mycotoxins on maize in various maize growing areas in Hungary in 2010 and 2011 years after harvest. Surface-sterilized cereal seeds were placed on selective media, and the isolated fungal strains were identified using morphological methods. 81.94% and 14.33% of the samples were found to be contaminated with potentially toxigenic isolates in 2010 and 2011, respectively. Species identifications of selected isolates have been carried out using sequence-based methods. Regarding *Fusarium* species, in 2010, when the weather was rainy, *F. graminearum* and *F. subglutinans* dominated, while in 2011 with hot and dry summer *F. verticillioides* was the predominant species identified. *F. culmorum* could not be detected in any of the samples. Regarding Aspergilli, several *Aspergillus flavus* isolates were identified, which are potential aflatoxin producers. Besides, other mycotoxin producer species were also isolated, including black Aspergilli which potentially produce ochratoxins and fumonisins, and *A. clavatus*, which produces patulin. Other genera (*Alternaria, Nigrospora, Epicoccum, Cladosporium*) were found in smaller proportions. Besides, the protective maize endophyte *Acremonium zeae* was also identified in some of the samples. Further studies are in progress to examine the mycotoxin producing abilities of the isolates, mycotoxin content of the maize samples, and the applicability of *Acremonium zeae* isolates for lowering fungal burden and mycotoxin contamination of maize.

Keywords: maize, mycotoxins, aflatoxins, fumonisins, *Fusarium, Aspergillus*

INTRODUCTION

Maize is among the most important ingredients of feed formulations worldwide. *Fusarium* species are among the most important mycotoxin producing pathogens of maize. These species cause several diseases on maize including ear rot and stalk rot (Figure 1), and contaminate maize kernel with various mycotoxins including trichothecenes, zearalenone and fumonisins. The most important maize pathogens are *F. graminearum, F. culmorum, F. verticillioides, F. proliferatum* and *F. subglutinans*. Besides, although not considered to be major causes of plant disease, *Aspergillus* and *Penicillium* species may also be responsible for several disorders in various plants including maize, and most importantly cause mycotoxin contamination [10]. The most notorious plant pathogens are black Aspergilli and *A. flavus* which may cause ear rot and are also important as postharvest pathogens. In contrast with specialized plant pathogens such as powdery mildews, rusts or most *Fusarium* species, these species are opportunistic pathogens without host specialization as proved in *A. flavus* [10]. The most important aspect of food and feed spoilage caused by these organisms is the formation of mycotoxins, which may have harmful effects on human and animal health. Several mycotoxins produced by Aspergilli or Penicillia have been identified in maize in previous studies, the economically most important of which are aflatoxins, ochratoxins and fumonisins [10].
In this study, we examined the mycobiota of maize samples collected in 2010 and 2011 from various maize growing regions of Hungary using morphologhical and sequence-based identification methods.

Figure 1. Fusarium ear rot of maize.

MATERIAL AND METHODS

Sample collection

The samples were collected in 9 and 10 maize growing regions in 2010 and 2011, respectively. Maize grains were surface sterilized using ethanol, and plated onto dichloran rose bengal agar media [1]. Plates were incubated at 25°C in darkness and monitored periodically for characteristic mycelium growing from the kernels. Single colonies were purified and transferred to malt extract agar (MEA) media. Isolates were subcultured as single conidia on MEA, Czapek-yeast extract agar (CYA) and potato dextrose agar (PDA) plates [8].

Identification of fungal isolates

Morphological identification of fungal isolates came from maize grains have been done according to standard textbooks and monographs [2, 6, 8]. For sequence based identification, the cultures used for the molecular studies were grown on malt peptone broth for 2 days, and DNA was extracted from the mycelia using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Parts of the ITS, Tri101 and calmodulin genes were amplified and sequenced as described previously [3, 5]. Sequences were compared using nucleotide-nucleotide BLAST (blastn) with default settings (http://blast.ncbi.nlm.nih.gov) to the Genbank database, and to our own sequence database. Species identifications were determined from the lowest expect value of the BLAST output.

RESULTS AND DISCUSSION

The overall fungal contamination rate of the samples was 81.94% in 2010, while only 14.33% of the samples were found to be contaminated in 2011 (Table 1). The lower contamination rate observed in 2011 is possibly due to the weather conditions. While the summer was rainy in 2010, it was dry and hot in 2011. Possibly due to the humid weather conditions, Penicillia were frequently isolated from the samples collected in 2010, while this genus was virtually absent in the samples collected in 2011. On the other hand, *Aspergillus* infection rates were higher in 2011 than in 2010. The higher prevalence of Aspergilli might have been caused by the warmer weather conditions in 2011, as these species (e.g. *A. flavus* or black Aspergilli) prefer higher temperatures. However, Fusaria were present in the samples in both years in large proportions (Table 1).
The number of primary isolates of each sample was restricted upon the grounds of colony and microscopic features and only the diverging ones were maintained for further investigations. 340 and 90 isolates were recovered in 2010 and 2011, respectively.

Table 1. Occurrence of mycotoxigenic fungi on maize in Hungary in 2010-2011

Year	Average infection of grains (%)	% of isolated fungal strains (based on sequence-based identification)			
		***Aspergillus* sp.**	***Penicillium* sp.**	***Fusarium* sp.**	**other genera**
2010	81.94	2.02	27.56	70.30	0.12
2011	14.33	8.27	0.00	60.25	31.48

Occurrence of Fusaria in maize kernel

In 2010, when the weather was rainy, *F. graminearum* and *F. subglutinans* dominated, while in 2011 with hot and dry summer *F. verticillioides* was the predominant species identified. *F. culmorum* could not be detected in any of the samples, in contrast with the results of the previous 1977 survey [4]. *F. graminearum* favors higher temperatures than *F. culmorum* and the observed shift might be an indication of climate change. However, *F. proliferatum* was detected in both years. Differences could not be observed in the species distribution of Fusaria in different locations (data not shown). Besides, *F. sporotrichioides* and *F. oxysporum* were also identified in some locations. Regarding the species distribution of *F. graminearum sensu lato* isolated from Hungarian maize, all isolates proved to belong to *F. graminearum sensu stricto* based on sequence analysis of the Tri101 gene of the isolates (data not shown). *Fusarium boothii* or *F. meridionale* could not be identified, in contrast with studies carried out in other countries including South Africa and Argentina [7].
Fumonisin and DON content of the maize samples was also analyzed using HPLC-MS. All samples were contaminated by these toxins under the EU limit (data not shown).

Occurrence of Aspergilli and Penicillia in maize kernel

Among the examined samples, several ones were found to be contaminated by members of section *Flavi* of the genus *Aspergillus* based on colony morphology and microscopic features. Although several *Aspergillus* species have been identified recently which are able to produce aflatoxins, *A. flavus, A. parasiticus* and *A. nomius* are the economically most important species regarding aflatoxin contamination of agricultural products [9]. These species can readily be distinguished using sequence analysis of part of their β-tubulin or calmodulin genes [9]. Species assignment of the isolates was carried out using partial sequence analysis of their calmodulin gene. All isolates assigned to section *Flavi*

based on morphological features were found to belong to the *A. flavus* species based on sequence data.
Besides *A. flavus*, several other potential mycotoxin producers were identified in the samples. The patulin producer *A. clavatus* and black Aspergilli able to produce both ochratoxins and fumonisins were recovered from several samples. Among black Aspergilli, *A. niger* was identified most frequently (41 isolates), although *A. tubingensis* (10 isolates) was also isolated from some samples. Regarding Penicillia, several mycotoxin producers were identified (e.g. *P. crustosum, P. brevicompactum, P. chrysogenum* and *P. viridicatum*). Other potentially mycotoxigenic genera (*Alternaria, Nigrospora, Epicoccum, Cladosporium*) were found in smaller proportions (data not shown). Besides, the protective maize endophyte *Acremonium zeae* was also identified in some of the samples for the first time in Central Europe [11]. This species has been shown to inhibit infection and mycotoxin accumulation caused by Fusaria and *A. flavus* by producing the antibiotic compounds pyrrocidins [11].
Aflatoxin content of the samples was analyzed using HPLC-MS. None of the samples were found to be contaminated by any of the aflatoxins (B_1, B_2, G_1, G_2). Examination of ochratoxin content of the samples is in progress.

CONCLUSIONS

During a survey of mycotoxin producing molds in Hungarian maize samples in 2010 and 2011, 81.94% and 14.33% of the samples were found to be contaminated with potentially toxigenic isolates, respectively. Regarding *Fusarium* species, in 2010, when the weather was rainy, *F. graminearum* and *F. subglutinans* dominated, while in 2011 with hot and dry summer *F. verticillioides* was the predominant species identified*. F. culmorum* could not be detected in any of the samples. Among Aspergilli, several *Aspergillus flavus* isolates were identified, which are potential aflatoxin producers. Besides, other mycotoxin producing species, including black Aspergilli which potentially produce ochratoxins and fumonisins, and *Penicillium* species producing a range of mycotoxins have also been identified. The most contaminated samples came from 2010, possibly due to the rainy, humid weather conditions. Samples came from 2011 were found to be infected less severely, but the *Aspergillus* infection was higher than in the previous year. Further studies are in progress to examine the mycotoxin producing abilities of the isolates, mycotoxin content of the maize samples, and the applicability of *Acremonium zeae* isolates for lowering fungal infection and mycotoxin contamination of maize.

ACKNOWLEDGEMENTS

This work was supported by OTKA grant Nos. K84122 and K84077, and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (B. Tóth). The project is co-financed by the European Union through the Hungary-

Serbia IPA Cross-border Co-operation Programme (ToxFreeFeed, HU-SRB/1002/122/062).

REFERENCES

1. **King, A.D.Jr., Hocking, A.D., Pitt, J.I.:** *Dichloran-rose bengal medium for enumeration and isolation of molds from foods*, Appl. Environ. Microbiol., 37 (1979), 959–964.
2. **Leslie, J.F., Summerell, B.A.:** *The Fusarium Laboratory Manual.* Blackwell Publishing Professional, Ames, USA, 2006, p. 388.
3. **Malihipour, A.:** *Genetic analysis of resistance to Fusarium head blight in wheat (Triticum spp.) using phenotypic characters and molecular markers,* Ph.D. Thesis, University of Manitoba, Winnipeg, Canada, 2010.
4. **Mesterházy, Á., Vojtovics, M.:** *A kukorica Fusarium spp. okozta fertőzöttségének vizsgálata 1972-1975-ben (Rate of Fusarium spp. infection in maize in 1972-1975),* Növénytermelés 26 (1977), 367-378.[in Hungarian]
5. **Pildain, M.B., Frisvad, J.C., Vaamonde, G., Cabral, D., Varga, J., Samson, R.A.:** *Two novel aflatoxin-producing Aspergillus species from Argentinean peanuts,* Int. J. Syst. Evol. Microbiol., 58 (2008), 725-735.
6. **Raper, K.B., Fennell D.I.:** *The genus Aspergillus*. Williams and Wilkins, Baltimore, USA, 1965, p. 686.
7. **Sampietro, D.A., Díaz, C.G., Gonzalez, V., Vattuone, M.A., Ploper, L.D., Catalan, C.A., Ward, T.J.:** *Species diversity and toxigenic potential of Fusarium graminearum complex isolates from maize fields in northwest Argentina,* Int. J. Food Microbiol. 145 (2011), 359-264.
8. **Samson, R.A., Hoekstra, E.S., Frisvad, J.C.:** *Introduction to Food- and Airborne Fungi,* 7th edition. CBS Fungal Biodiversity, Center, Utrecht, Netherlands, 2004, p. 389.
9. **Varga, J., Frisvad, J.C., Samson, R.A.:** *A reappraisal of fungi producing aflatoxins,* World Mycotoxin J. 2 (2009), 263-277.
10. **Varga, J., Tóth, B., Mesterházy, Á., Téren, J., Fazekas, B.:** *Mycotoxigenic fungi and mycotoxins in foods and feeds in Hungary,* in: An overview on toxigenic fungi and mycotoxins in Europe. Eds. **A. Logrieco, A. Visconti.** Kluwer Academic Publishers, Amsterdam, Netherlands, 2004, p. 123-139.
11. **Wicklow, D.T., Roth, S., Deyrup, S.T., Gloer, J.B.:** *A protective endophyte of maize: Acremonium zeae antibiotics inhibitory to Aspergillus flavus and Fusarium verticillioides,* Mycol. Res. 109 (2005), 610–618.