UDC 575.
https://doi.org/10.2298/GENSR1901357N
Original scientific paper

MAIZE RESISTANCE TO EAR ROT CAUSED BY *Aspergillus parasiticus*

Milica NIKOLIĆ, Iva SAVIĆ, Ana NIKOLIĆ, Goran STANKOVIĆ, Nenad DELIĆ, Snežana MLADENOVIĆ DRINIĆ, Slavica STANKOVIĆ

Maize Research Institute, Zemun Polje, Belgrade, Serbia

Nikolić M., I. Savić, A. Nikolić, G. Stanković, N. Delić, S. Mladenović Drinić, S. Stanković (2019): *Maize resistance to ear rot caused by Aspergillus parasiticus.*- Genetika, Vol 51, No.1, 357-363.

The fungus *Aspergillus parasiticus* produces aflatoxins, the most important group of mycotoxins considering their potential toxicity that may cause cancer in humans. Prevention is the most important and economically most beneficial practice in the reduction of fungal growth and mycotoxin production. Due to that, the development of resistant maize genotypes is the most effective method. The aim of the present study was to analyse the resistance of maize hybrids to pathogenic and toxigenic *A. parasiticus* isolates originating from maize in Serbia. Hybrids used belong to three FAO maturity groups and showed a high level of resistance to *A. parasiticus* species. A combination of prevention management strategies and good grain management at harvest can lessen the impact of *Aspergillus* ear rot on yield and grain quality.

Keywords: tolerance, genotypes, *A. parasiticus*

INTRODUCTION

In terms of distribution and economic importance of aflatoxigenic species, only species belonging to the section Flavi are significantly related to health safety of food and food products. *Aspergillus flavus* and *A. parasiticus* are representatives of aflatoxigenic species that naturally occur in food products around the world. These are related fungi that may contaminate primary agricultural products in the field, at harvest, in storages and during processing (DIENER *et al*., 1987).

Aspergillus contamination has been rare under agroecological conditions of cereal-growing areas of Serbia. However, based on changes in climatic factors, such as the occurrence of high temperatures and prolonged droughts, which favour the increased frequency of

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

Aspergillus spp., it has assumed that this pathogen may be a problem in Serbia. The species *A. parasiticus* was isolated from maize grain for the first time in Serbia in the growing season of 2012 (STANKOVIĆ *et al*., 2015). Furthermore, the isolates obtained from wheat grains were identified as *A. parasiticus*, for the first time under climatic conditions of Serbia in 2017 (NIKOLIĆ *et al*., 2018).

Beside direct losses from infections of maize kernels, maize is considered one of the most susceptible crops to mycotoxins worldwide. *A. parasiticus* produces aflatoxins, which are the most important group of mycotoxins considering their potential carcinogenicity and significant and longstanding problems that they cause in humans and animals. *A. parasiticus* is capable of synthesizing four aflatoxins B_1, B_2, G_1, and G_2, but not cyclopiazonic acid (AMAIKE and KELLER, 2011). Due to extremely harmful effects of these mycotoxins, their amounts are legally regulated in food and food products in Europe and our country (KOS, 2015).

Strategies for the reduction or elimination of mycotoxins are known. Prevention is the most important and economically most beneficial plan to reduce the fungal growth and mycotoxin production. One of the most effective prevention method is the development of resistant maize genotypes.

Due to both, economic damages on maize kernels caused by *A. parasiticus* and potential contamination caused by aflatoxins, the aim of the present study was to analyse the resistance of maize hybrids to pathogenic and toxigenic *A. parasiticus* isolates originated from maize in Serbia.

MATERIALS AND METHODS

Ten *A. parasiticus* isolates, isolated from maize kernels in Serbia were used in this study. Susceptibility of three maize hybrids to ear rot, caused by *A. parasiticus*, was evaluated in field trials during 2016 and 2017. A selected group of 20 hybrids was inoculated by the injection of fungal spore suspension into the silk channel. After harvest, ears were rated for *Aspergillus* rot and evaluated for levels of aflatoxin contamination.

Preparation of spore suspension

A potato dextrose agar (PDA, Biolife, Milan, Italy) was used to culture *A. parasiticus* isolates at the temperature of 25°C in the dark. The spore suspension was prepared by flooding the 7-day old mycelia with 10 ml sterile distilled water and 0.01% Tween 20 added. The haemocytometer was used to adjust the suspension concentration to approximately $3x10^7$ spores ml^{-1}.

Inoculation

A method developed by REID *et al.* (1996) was used for artificial inoculations. Inoculation was carried out 2 days after 50% of plants reached the silking stage. An injury on the upper cob was made with a syringe fitted with a needle immediately prior to inoculation, and 2 ml of inoculum was injected per cob. Positive control cobs were inoculated by applying the equal amount of *A. flavus* and negative control cobs were inoculated by applying the equal amount of sterile distilled water. Five cobs in four replicates were inoculated with such prepared conidial suspension (total of 20 cobs per isolate). Visual rating was performed according to REID *et al.* (1996), on the 1-7 scale (1 - complete absence of symptoms; 7 - 76-100% infected kernels).

RESULTS AND DISCUSSION

Visual evaluation of disease severity

Virulence of isolates of this fungus is reflected through their capability to overcome defence responses of host plants, to infect and colonise the plant tissue. Symptoms were observed during the harvest.

The results indicated a different tolerance, which was assessed on the basis of the degree of infection according to the 1-7 scale at maize harvest. This scale included the follow rates:

Degree 1 – cobs without symptoms; Degree 2 – (1-3%); Degree 3 – (4-10%); Degree 4 – (11-25%); Degree 5 – (26-50%); Degree 6 – (51-75%); Degree 7 – (76-100% of the infected cob).

Under conditions of artificial inoculation, during the first trial year (2016), the tested isolates exhibited an average degree of the disease intensity in the range of 1.2-2.15 on hybrids of the FAO 300. The lowest, i.e. highest pathogenicity was observed in the isolates MRI 10Ap (average degree: 1.2), i.e. MRI 3937 (*A. flavus*) (average degree: 2.6), which was used as a positive control in the test, respectively. In addition, the highest average pathogenicity was obtained by the isolate MRI 1Ap. In the hybrids of the FAO 500, the investigated isolates showed the mean intensity of the infection in the range of 1.3-2.5. The isolates MRI 1Ap and MRI 9Ap isolates exhibited the lowest pathogenicity, while the highest one (average degree: 2.55) was recorded in the isolate MRI 3937 (*A. flavus*), and then in the MRI 4Ap isolate (Table 1). The investigated isolates showed the mean intensity of the infection in the range of 1.05-1.45 (FAO 800 hybrids). The MRI 5Ap and MRI 8Ap isolates showed the least pathogenicity, while the largest pathogenicity (average degree: 2.05) was detected in the MRI 3937 (*A. flavus*) isolate, and then in the MRI 2Ap isolate (Table 1).

The average assessment ratings of the infection intensity in the second year of the study, 2017, ranged from 1.15 to 1.9 in the hybrids of FAO 300. The lowest and highest pathogenicity was observed in the isolates MRI 8Ap, and MRI 1Ap and MRI 9Ap, respectively. The mean intensity of the infection in hybrids of FAO 500 ranged from 1.4 to 2.2. The MRI 6Ap isolate exhibited the lowest pathogenicity, while the highest (average score: 2.80) was observed in the MRI 3937 (*A. flavus*) isolate, then in MRI 2Ap. The mean intensity of the infection in hybrids of FAO 800 ranged from 1.05 to 2.0. The MRI 1Ap isolate exhibited the lowest pathogenicity, while the highest (average score: 2.45) was observed in the MRI 3937 (*A. flavus*) isolate, then in isolate MRI 9Ap (Table 1).

Based on the obtained results, it can be concluded that the highest tolerance was recorded in the FAO 800 hybrid, while the FAO 500 hybrid was the least resistant in the first year of study. During the second year of testing (2017), the FAO 300 hybrid was the most tolerant, while the least resistant was, also, the FAO 500. The overall observation of all three hybrids showed a high level of resistance to *A. parasiticus* species. The highest number (83%) of tested hybrids showed moderate resistance to this pathogenic species (Table 1).

The comparison of the average degrees of the infection during the two examined years (average degree for 2016: 1.47; average degree for 2017: 1.63), showed that the isolates did not exhibit a uniform pathogenicity (Table 1).

Table 1. Resistance assessment ratings of maize hybrids from FAO maturity groups 300, 500 and 800 in years 2016 and 2017 according to Aspergillus ear rot

Isolates	Rating of resistance					
	2016			2017		
	FAO 300	FAO 500	FAO 800	FAO 300	FAO 500	FAO 800
MRI 1Ap	2.15	1.3	1.25	1.9	2.1	1.05
MRI 2Ap	1.4	2.0	1.45	1.3	2.2	1.65
MRI 3Ap	1.5	1.4	1.15	1.65	1.8	1.7
MRI 4Ap	1.35	2.5	1.4	1.3	1.65	1.75
MRI 5Ap	1.6	2.05	1.05	1.3	1.95	1.95
MRI 6Ap	1.65	1.6	1.25	1.4	1.4	1.55
MRI 7Ap	1.45	1.75	1.05	1.5	1.6	1.4
MRI 8Ap	1.35	1.55	1.05	1.15	1.85	1.75
MRI 9Ap	1.3	1.3	1.1	1.9	1.55	2.0
MRI 10Ap	1.2	1.7	1.35	1.4	1.65	1.65
Average	1.5	1.72	1.21	1.48	1.78	1.65
Average per year		1.47			1.63	

These results agree with results obtained on variations in pathogenicity gained by GIORNI (2007). Results indicate that the average pathogenicity of isolates was 2.45 in the experiments carried out in 2005, while in those performed in 2006, aggressiveness of isolates was lower and amounted to 1.30. Pathogenicity varied from 1 to 5.1 (2005), and from 1 to 1.8 (2006). Different weather conditions are thought to have significantly affected extreme changes in the occurrence of the infection during the years of investigation (2005, 2006).

Researching the pathogenicity of the isolates of the genera *Fusarium* and *Aspergillus*, BAGI *et al.* (2012) found that the average intensity of *A. flavus* isolates was 1.81 on the various maize hybrids examined in a two-year experiment, indicating their poor pathogenicity.

Studying the new concept of food safety protection from the species *Fusarium* and *Aspergillus*, SZABO *et al*. (2018) noted that the infection intensity of *A. flavus* in the corn hybrid resistance tests was 0.3-1.52, with an average value of 0.78.

The results of NIKOLIĆ (2019) also demonstrated the pathogenicity of *A. parasiticus* isolates lower intensity. Comparative overview of the average estimate of infection during the two examined years was 1.46 (2016), and 1.71 (2017).

CONCLUSIONS

Based on the gained results it can be concluded that the FAO 800 hybrid was the most tolerant, while the FAO 500 hybrid was the least resistant, in the first year of study (2016). During the second year of testing (2017), the FAO 300 hybrid was the most tolerant, while the FAO 500 hybrid was the least resistant. All three hybrids showed a high level of resistance to *A. parasiticus* species. The highest number of investigated hybrids showed moderate resistance to this pathogenic species.

This risk would be even more certain if global climate changes continue and thus provide the development of this pathogen. The ability to estimate climate changes and the comparison of that between these changes and fungal infection and subsequent aflatoxins contamination will help in predicting and dealing with this emerging risk.

A combination of preventative management tactics and good grain management at harvest can lessen the impact of *Aspergillus* ear rot on yield and grain quality. The most important preventive measure is cultivation of resistant genotypes. For this reason, continuous research of the susceptibility of local hybrids to toxigenic fungal species is required.

ACKNOWLEDGEMENTS

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

Received, November 07th, 2018
Accepted February 18th, 2019

REFERENCES

ABBAS, H.K., W.T., SHIER, B.W., HORN, M.A., WEAVER (2004): Cultural methods for aflatoxin detection. Toxin Rev., *23*: 295–315.

AMAIKE, S. and N.P., KELLER (2011): *Aspergillus flavus*. Annu. Rev. of Phytopathol., *49*: 107–133.

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

BAGI, F., V., STOJŠIN, D., BUDAKOV, A., MESTERHAZY, J., VARGA, J., VUČKOVIĆ, B., TOTH (2012): Pathogenicity of *Fusarium* spp. and *Aspergillus flavus* on maize ears. In: International Symposium: Current Trends in Plant Protection, Belgrade, 25-28 September, 282–286. ISBN: 978-86-910951-1-6.

BAQUIÃO, A.C., M.M., OLIVEIRA, T.A., REIS, P., ZORZETE, D., DINIZ ATAYDE, B., CORREA (2013): Polyphasic approach to the identification of *Aspergillus* section Flavi isolated from Brazil nuts. Food Chem., *139*: 1127–1132.

BARROS, G.G., A.M., TORRES, M.I., RODRIGUEZ, S.N., CHULZE (2006): Genetic diversity within *Aspergillus flavus* strains isolated from peanut-cropped soils in Argentina. Soil Biol. Biochem., *38*: 145–152.

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. of Phytopathol., *25*: 249–270.

DONNER, M., J., ATEHNKENG, R.A., SIKORA, R., BANDYOPADHYAY, P.J., COTTY (2009): Distribution of *Aspergillus* section *Flavi* in soils of maize fields in three agroecological zones of Nigeria. Soil Biol. Biochem., *41*: 37–44.

GIORNI, P. (2007): Impact on Environmental and Plant actors on *Aspergillus* section Flavi isolated from maize in Italy. 205p. Ph.D. Thesis. Faculty of Medicine and Biosciences, Cranfield Univesity, Italy.

HORN, B.W., R.L., GREENE, V.S., SOBOLEV, J.W., DORNER, J.H., POWELL, R.C., LAYTON (1996): Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus, A. parasiticus,* and *A. tamarii.* Mycologia, *88*: 574–587.

KOS, J. (2009): Aflatoksini: analiza pojave, procena rizika i optimizacija metodologije određivanja u kukuruzu i mleku. PhD thesis. University of Novi Sad, Faculty of Technical Sciences, Novi Sad, Serbia.

NIKOLIĆ, M. (2019): *Aspergillus parasiticus* – toksigena vrsta gljive na zrnu kukuruza u proizvodnim uslovima Srbije. PhD thesis. University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia.

NIKOLIĆ, M., S., STANKOVIĆ, I., SAVIĆ (2017): Comparison of methods for determination of the toxigenic potential of *Aspergillus parasiticus* Speare and *Aspergillus flavus* Link isolated from maize. In: *6th International Scientific*

Meeting: Mycology, Mycotoxicology and Mycoses, September 27-29, 2017, Novi Sad, Serbia, published in *Matica Srpska: J. Nat. Sci., 133*: 95–104.

NIKOLIĆ, M., A., NIKOLIĆ, M., JAUKOVIĆ, I., SAVIĆ, T., PETROVIĆ, F., BAGI, S., STANKOVIĆ (2018): Differentiation between *Aspergillus flavus* and *Aspergillus parasiticus* isolates originated from wheat. Genetika, *50*: 143–150.

OKOTH, S., B., NYONGESA, V., AYUGI, E., KANG'ETHE, H., KORHONEN, V., JOUTSJOKI (2012): Toxigenic potential of *Aspergillus* species occurring on maize kernels from two agro-ecological zones in Kenya. Toxins, *4*: 991–1007.

OKUN, O.D., M.K., FATHIYA, M.M., GEOFFREY, J.N., JOSEPH, O.O., FIDELIS, O.Y., MOSES, U.K., EUCHARIA (2015): Distribution of indigenous strains of atoxigenic and toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* in maize and peanuts agro-ecological zones of Kenya. Agriculture and Food Security, *4*:14.

PITT, J.I. and A.D., HOCKING (2009): *Aspergillus* and Related Teleomorphs. In: *Fungi and Food Spoilage*. *Springer*, Boston, MA. p. 275–337.

REID, L.M., R.I., HAMILTON, D.E., MATHER (1996): Screening Maize for Resistance to Gibberella Ear Rot. Research Branch Agriculture and Agri-Food Canada, Technical Bulleten 5E.

RODRIGUES, P. (2011): Mycobiota and aflatoxigenic profile of Portuguese almonds and chestnuts from production to commercialization. 279p. Ph.D. thesis, Universidade do Minho, Braga, Portugal.

RODRIGUES, P., A., VENÂNCIO, Z., 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.

STANKOVIĆ, S., J., LEVIĆ, M., NIKOLIĆ, V., KRNJAJA, M., JAUKOVIĆ (2015): Prvi nalaz *Aspergillus parasiticus* u proizvodnji kukuruza u Srbiji. In: *Savetovanje o zaštiti bilja*, in November in Zlatibor, Serbia. p. 32–33.

SZABO, B., B., TOTH, E., TOTH TOLDINE, M., VARGA, N., KOVACS, J., VARGA, S., KOCSUBE, A., PALAGYI, F., BAGI, D., BUDAKOV, V., STOJŠIN, S., LAZIĆ, M., BODROŽA-SOLAROV, R., ČOLOVIĆ, G., BEKAVAC, B., PURAR, DJ., JOCKOVIĆ, A., MESTERHÁZY (2018): A new concept to secure food safety standards against *Fusarium* species and *Aspergillus flavus* and their toxins in maize. Toxins, *10*(9): 372-396.

VAAMONDE, G., A., PATRIARCA, V.F., PINTO, R., COMERIA, C., DEGROSSI (2003): Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section Flavi from different substrates in Argentina. Int. J. Food Microbiol., *88*: 79–84.

OTPORNOST KUKURUZA NA TRULEŽ KLIPA KOJU IZAZIVA *Aspergillus parasiticus*

Milica NIKOLIĆ, Iva SAVIĆ, Ana NIKOLIĆ, Goran STANKOVIĆ, Nenad DELIĆ, Snežana MLADENOVIĆ DRINIĆ, Slavica STANKOVIĆ

Maize Research Institute, Zemun Polje, Belgrade, Serbia

Izvod

Toksigena vrsta gljive *Aspergillus parasiticus* stvara aflatoksine, najvažniju grupu mikotoksina s obzirom na njihovu potencijalnu toksičnost koja može izazvati rak kod ljudi. Preventivne mere su najvažnije i ekonomski najznačajnije mere koje se primenjuju u cilju sprečavanja i razvoja toksigenih vrsta gljiva i stvaranja mikotoksina. U tom smislu, stvaranje otpornih genotipova kukuruza je najefikasniji metod. Cilj ovog rada bio je da se utvrdi otpornost hibrida kukuruza različitih FAO grupa zrenja na patogene i toksigene izolate *A. parasiticus* poreklom iz zrna kukuruza u Srbiji. Utvrđeno je da su svi ispitivani hibridi ispoljili visok stepen otpornosti na vrstu *A. parasiticus*. Kombinacija preventivnih mera, kao i odgovarajući uslovi skladištenja zrna mogu da ublaže uticaj vrsta roda *Aspergillus* na prinos i kvalitet zrna.

Primljeno 07.XI.2018.
Odobreno 18. II. 2019.