Review on Agriculture and Rural Development 2021 vol. 10 (1-2) ISSN 2677-0792
DOI: 10.14232/rard.2021.1-2.65-70

NATURAL *FUSARIUM* TOXIN CONTAMINATION OF WHEAT IN SOUTHERN PART OF HUNGARY

ATTILA BERÉNYI[1*], ÁKOS MESTERHÁZY Á.[1], LÁSZLÓ CSEUZ[1], CSABA VÁGVÖLGYI[2], TAMÁS MESZLÉNYI[1], BEÁTA TÓTH[1]

[1] Cereal Research Non Profit Ltd. 6726, Szeged, Hungary
[2] Department of Microbiology, Faculty of Science and Informatics, University of Szeged, 6726, Szeged, Hungary
*Corresponding author: attila.berenyi@gabonakutato.hu

ABSTRACT
Fusarium head blight (FHB), primarily caused by a fungal plant pathogen *Fusarium graminearum*, is a devastating disease of wheat and other cereals. FHB reduces yield, but also the quality and feeding value of the crop. The fungus produces the mycotoxin deoxynivalenol (DON) that poses a significant threat to the health of domestic animals and humans. In 2019 *Fusarium* epidemic occurred not only the southern and eastern parts of Hungary, but affected other area of Central Europe. The mycotoxin patterns varied tremendously, depending on the geographic area, different resistance levels of wheat genotypes, weather and soil conditions and cropping factors. In this survey 192 samples of different bread wheat (*Triticum aestivum* L.) genotypes from three geographically different regions were tested for DON toxin contamination by HPLC-MS method. Average levels of mycotoxin contamination 3.80 mg/kg; it is three times higher than the European maximum limit for unprocessed cereals intended for human consumption. The content range is very wide (0.15 – 20.71 mg/kg), 76% of the samples exceeded the EU risk threshold level.

Keywords: *Fusarium* head blight, DON, *Fusarium* epidemic, HPLC, toxin

INTRODUCTION

Fusarium head blight (FHB), also known as scab, is an important fungal disease of wheat, barley and other cereals (MESTERHÁZY, 1984; MOSCHINI ET AL., 2001). FHB results not only reduction in yield, but also spoils quality and usability (SAYLER, 1998; GOSHWAMI AND-KISTLER, 2004). The main causes of FHB are *F. graminearum* and *F. culmorum* in Europe, but *F. graminearum* is also the predominant species in the United States (TÓTH ET AL., 2008; DOOHAN ET AL., 1998; XU ET AL., 2005). These fungus species can produce harmful secondary metabolites, called mycotoxins, which can cause food and feed safety risk The most important *Fusarium* mycotoxin is DON (deoxynivalenol) belongs to the type B trichothecenes family (DESJARDINS AND PROCTOR, 2007). DON and other trichothecenes (TERZI ET AL., 2014) can inhibit eukaryotic protein synthesis and can cause anorexia, diarrhea, vomiting, cell death, gastrointestinal function, immune suppression and can confuse the action of growth hormones (PESTKA, 2010). This is the reason that the EU and the countries of the world have set strict limit values for treated and untreated cereals and food (SELAMAT AND IQBAL, 2016). *Fusarium* sporulation and wheat head infection is favored by moist, warm conditions during flowering. The warming weather inhibits disease and toxin development, but there are years with moderate warm and wet flowering periods (like in 2019) supporting outbreak of heavy epidemics and toxin contamination (MORETTI ET AL., 2018). The FHB is most severe in warm and wet conditions during anthesis (MESTERHÁZY, 1995; PARRY ET AL., 1995). In 2019, weather conditions were favorable for *Fusarium* infections, in May the amount of precipitation was 134 mm, while rainfall was also in surplus through July. This significant precipitation level was the major precursor to *Fusarium* mold growth, so a strong *Fusarium* epidemic occurred in most

Review on Agriculture and Rural Development 2021 vol. 10 (1-2) ISSN 2677-0792
DOI: 10.14232/rard.2021.1-2.65-70

regions of wheat growing area of the country. In 2019, *Fusarium* infection caused enormous problems in the whole southern European region. The west Romanian and north Serbian territories showed similar picture in DON contamination (COTUNA ET AL., 2021) and this was the same on the Hungarian side of the border.

MATERIALS AND METHODS

Plant materials

Different bread wheat (*Trtiticum aestivum* L.) genotypes from two conventional breeding nurseries (192 samples) were tested for DON toxin contamination in 2019. Samples derived from the south part of Hungary near Szeged and Makó, the distance between the two locations is 31 km. The ninety-six tested wheat genotypes with wide genetic background were the same at both locations. Furthermore 24 wheat samples derived from Táplánszentkereszt (close to Szombathely). All the tested genotypes chose from breeding materials of the Cereal Research Non Profit Ltd. We examined four experimental block, every block contained twenty different species combination and four controls.

Toxin extraction and analysis

The samples were ground into flour using a laboratory mill Perten Laboratory Mill 3310, Perten Instruments, United Kingdom). Four ml of acetonitrile:water mixture (84:16) was weighed into centrifuge tubes to which 1-1 g flour samples were added. These were extracted on a vertical shaker (Stuart STR4 rotator, Stuart Equipment, United Kingdom) for 2.5 hours. The samples were then centrifuged at 4400 rpm for 10 min (Heraeus Megafuge 8, Thermo Fischer Scientific, United States). One ml of the supernatant was pressurized under high pressure through an alumina:activated carbon (20/1) SPE tube. 500 µl of the purified material was measured into vials and then evaporated. The residual solid was redissolved in acetonitrile:water (20:80) and the sample was homogenized by vortexing and sonication for 5 min. Subsequently, the DON toxin content of the sample was determined using an Agilent 1260 HPLC equipped with DAD instrument (Agilent, United States). A Zorbax SB-Aq (3.5 µm, 4.6 x 50 mm) HPLC column (Agilent, United States) with a ZorbaxSB-Aq (3.5 µm, 4.6 x 12.5 mm) pre-column was used to separate the sample components. Five µl of the prepared samples was injected onto the HPLC column. The flow rate was one ml/min. The column temperature was maintained at 40 °C. Detection was performed at 219 nm. All the used chemicals purchased from Sigma Aldrich Ltd. Correlation and regression analyses based on the Excel built-in functions.

RESULTS

In case of samples derived from Szeged the average DON toxin contamination was 1.84 mg/kg, this value is higher than the EU threshold level (1.25 mg/kg). From the genotypes we examined more than 55% had higher DON toxin content than the EU limit value. The content range is very wide, from 0.15 mg/kg to 8.02 mg/kg (*Figure 1.*). The controls (orange lines on the figures) had 1.44 mg/kg average DON contamination. 46% of the genotypes had lower toxin content than the average control value. Only 2% of the examined genotypes had lower DON contamination than those of the most resistant control species.

Review on Agriculture and Rural Development 2021 vol. 10 (1-2) ISSN 2677-0792
DOI: 10.14232/rard.2021.1-2.65-70

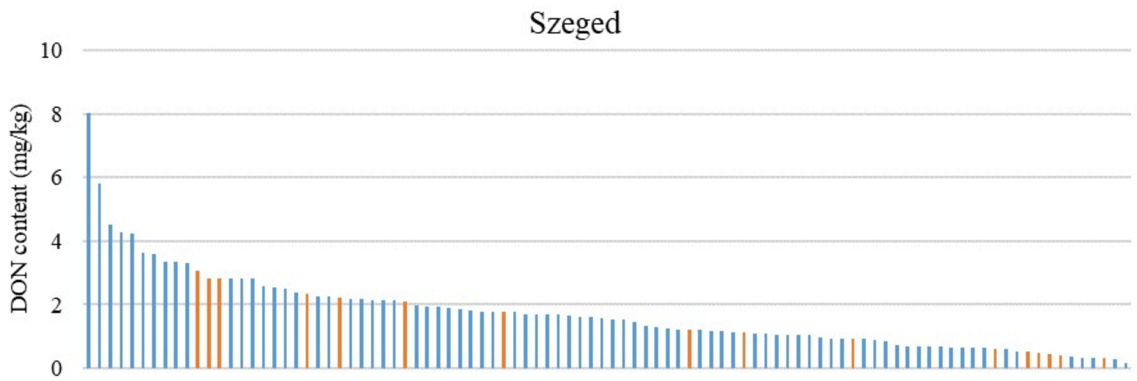

Figure 1. DON toxin contamination of different genotypes from Szeged, Csongrád-Csanád county, 2019

In Makó the DON contamination of the samples was much higher than in Szeged. The average value was 5.77 mg/kg, which is almost five times higher than the EU limit. (*Figure 2.*) Furthermore, in Makó only 3.13% of the examined genotypes had lower DON toxin contamination than the EU risk threshold level, and 6.25% had lower toxin content than the most resistant control. Average control contamination was 3.63 mg/kg.

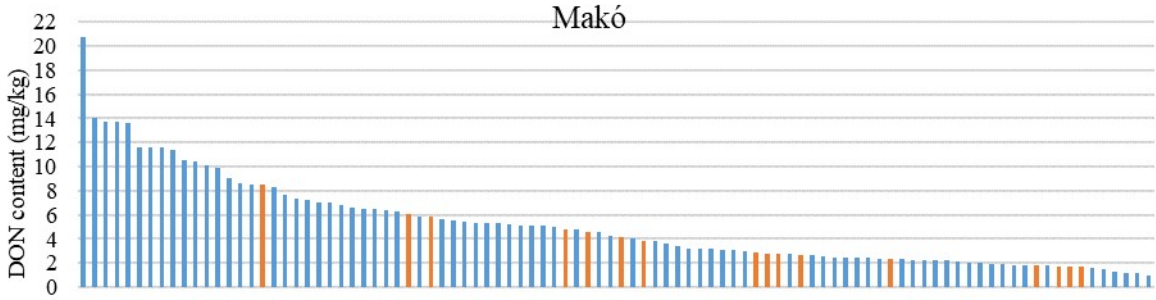

Figure 2. DON toxin contamination of different genotypes from Makó, Csongrád-Csanád county, 2019

The possible reason for the DON content differences between samples arrived from Szeged and Makó, the uneven precipitation distribution. In May the rainfall level was the same (~134 mm) in the two locations, but in June the precipitation level was 206 mm in Makó, and only the half of this value in Szeged despite of the small distance. For us the most important information was the several genotypes that were low toxin contaminated in both tests (*Figure 3.*). From these results, the good yielding lines will be identified and also genotypes having better quality and resistance also to other diseases like leaf rust, leaf spots, etc. It is very important that there are exploitable differences between lines not bred with FHB resistance, which can develop the portfolio of the Cereal Research Institute.

Review on Agriculture and Rural Development 2021 vol. 10 (1-2) ISSN 2677-0792
DOI: 10.14232/rard.2021.1-2.65-70

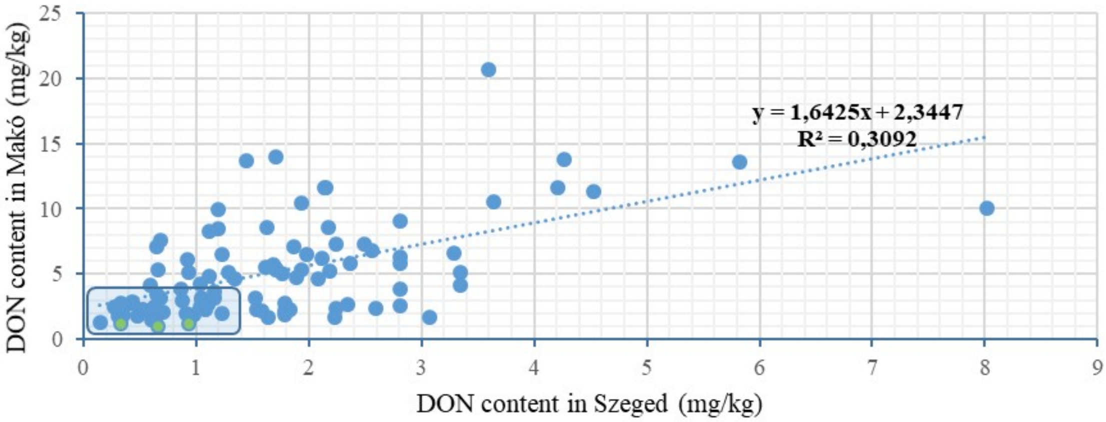

Figure 3. The correlation and regression of the DON toxin contamination in Szeged and Makó. Green spots shows the genotypes which had lower toxin contamination than the EU limit in both locations. The box shows those genotypes which had lower toxin content than the controls average contamination**.**

In Táplánszentkereszt, which is located in the northwest part of Hungary, the average DON content was 1.5 mg/kg, and 50% of the examined genotypes had lower toxin level than the EU limit value (*Figure 4.*). 33% of the samples had lower toxin content than the most resistant control. The average toxin contamination was 1.92 mg/kg in case of the control species.

Figure 4. DON toxin contamination of different genotypes from Táplánszentkereszt, Vas county, 2019

DISCUSSION

In 2019, due to the favorable weather conditions, there was a remarkable Fusarium epidemic in the southern part of Hungary. The average internal *Fusarium spp.* infestation level was 22.9 for the whole country (CSERTÁN-HALÁSZ, 2019). Detailed values show that the infection level is not the same for all counties. As breeding stations of the Cereal Research Nonprofit Ltd. are in Csongrád-Csanád and Vas counties, we focused on the DON toxin content of samples derived from these regions. Based on the survey of National Food Chain Safety Office, in Csongrád-Csanád county the internal *Fusarium spp.* infestation level was 21.17%, till in Vas county, this value didn't reach the 5%. Although we can find counties close 50% values, mainly on the Great Plain.
The average DON toxin content is similar in Szeged and Táplánszentkereszt (1.84 and 1.5 mg/kg, respectively), but the internal *Fusarium* infestation levels are different in the two locations. These results confirm that we have to check the toxin contamination of the

cereal samples besides the phenotypical evaluation of the symptoms caused by *Fusarium*, because in some cases only slight correlation can be found between the two types of data. Furthermore large differences can be found between the three locations in same genotypes (*Figure 5.*). On the one hand the different weather conditions can explain the distinct toxin values. The different ecological and agro-technical factors indirectly also can influence the infection levels.

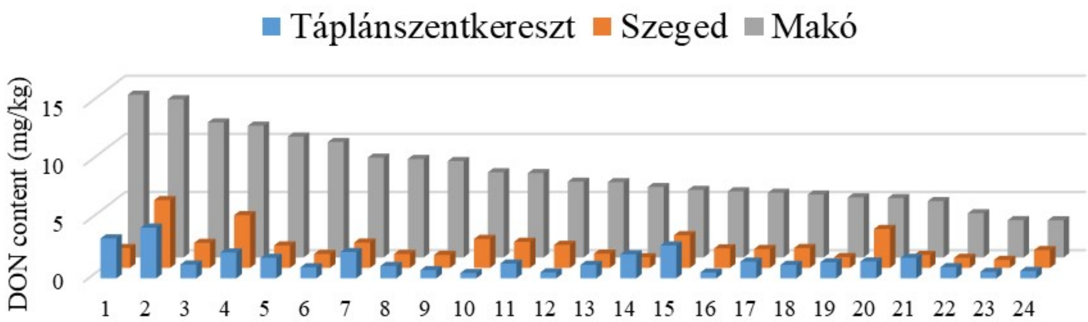

Figure 5. DON toxin contamination of different genotypes from Táplánszentkereszt, Szeged and Makó, 2019

We found only three genotypes on the two locations which had lower toxin contamination than the EU limit value and only two samples had lower toxin content than the most resistant control toxin level. It is essential to monitor the toxins in our cereals, because of the given circumstances, in case of same species there can be found extremely high differences. It is known that occurrence of natural infection is periodical, less predictable than the artificial inoculation results, but the data provide a useful feedback for the breeding work. It is important that wheat genotypes were identified with low DON contamination in both tests across two and three locations.

ACKNOWLEDGMENT

Prepared with the professional support of the doctoral student scholarship program of the co-operative doctoral program of the Ministry of Innovation and Technology financed from the National Research, Development and Innovation found. (1022317; KDP-17-4/PALY-2021)

The authors thank the support of the TKP2020-NKA-21 project.

REFERENCES

Cotuna, O., Paraschivu, M., Sărățeanu, V., Partal, E., Durău, C.C. (2021): Influence of *Fusarium graminearum* Infection on the Accumulation of Mycotoxins in Wheat Grains. *Preprints* 2021060429. DOI: 10.20944/preprints202106.0429.v1

Csertán-Halász, Á. (2019): A magyarországi őszi búza tételek belső *Fusarium*-fertőzöttség felmérésének eredményei 2019-ben. Agrofórum Online 2019.12.01.

Desjardins, A., Proctor, R. (2007): Molecular biology of Fusarium mycotoxins. International Journal of Food Microbiology 119: 47-50. DOI: 10.1016/j.ijfoodmicro.2007.07.024

Doohan, F. M., Parry, D. W., Jenkinson, P., and Nicholson, P. (1998): The use of species-specific PCR based assays to analyze *Fusarium* ear blight of wheat. Plant Pathology 47: 197-205. DOI:10.1046/J.1365-3059.1998.00218.X

Review on Agriculture and Rural Development 2021 vol. 10 (1-2) ISSN 2677-0792
DOI: 10.14232/rard.2021.1-2.65-70

Goswami, R. S., Kistler, H. C. (2004): Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5: 515-525. DOI:10.1111/j.1364-3703.2004.00252.x

Mesterházy, Á. (1984): Fusarium species of wheat in South Hungary, 1970–1983. Cereal Research Communications 12: 167-170.

Mesterházy, Á. (1995): Types and components of resistance to *Fusarium* head blight of wheat. Plant Breeding 5: 377-386. DOI:10.1111/j.1439-0523.1995.tb00816.x

Moretti, A., Pascale, M., Logrieco, A. F. (2018): Mycotoxin risks under a climate change scenario in Europe. Trends in Food Science and Technology 84: 38-40. DOI:10.1016/j.tifs.2018.03.008

Moschini, R. C., Pioli, R., Carmona, M., and Sacchi, O. (2001): Empirical predictions of wheat head blight in the northern Argentinean Pampas region. Crop Science 41: 1541-1545. DOI:10.2135/cropsci2001.4151541x

Sayler, T. (1998): Study: $2.6 billion, 501 million bushels lost to scab 1991-96. Prairie Grains 11.

Selamat J., Iqbal S. Z. (2016): Food Safety. Basic Concepts, Recent Issues, and Future Challenges. Springer International Publishing, Switzerland.

Parry, D. W., Jenkinson, P., and McLeod, L. (1995): *Fusarium* ear blight (scab) in small grains - A review. Plant Pathology 44: 207-238. DOI:10.1111/j.1365-3059.1995.tb02773.x

Pestka, J. (2010): Toxicological mechanisms and potential health effects of deoxynivalenol and nivalenol. World Mycotoxin Journal 3: 323-347. DOI:10.3920/WMJ2010.1247

Terzi, V., Tumino, G., Stanca, A. M., Morcia, C. (2014): Reducing the incidence of cereal head infection and mycotoxins in small grain cereal species. Journal of Cereal Science 59: 284-293. DOI**:** 10.1016/j.jcs.2013.10.005

Tóth, B., Kászonyi G., Bartók T., Varga J., Mesterházy Á. (2008): Common resistance of wheat to members of the *Fusarium graminearum* species complex and *F. culmorum*. Plant Breeding 127: 1-8. DOI: 10.1111/j.1439-0523.2008.01412.x

Xu, X.-M., Parry, D., Nicholson, P., Simpson, D., Edwards, S., Cooke, B., Doohan, F., Brennan, J., Monaghan, S., Moretti, A., Tocco, G., Mule, G., Hornok, L., Giczey, G., Tatnell, J. (2005): Predominance and association of pathogenic species causing *Fusarium* ear blight in wheat. European Journal of Plant Pathology 112: 143-154. DOI: 10.1007/s10658-005-2446-7