Biotechnology in Animal Husbandry 31 (1), p 123-131 , 2015 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 632.4'633.11
DOI: 10.2298/BAH1501123K

FUSARIUM INFECTION AND DEOXYNIVALENOL CONTAMINATION IN WINTER WHEAT

V. Krnjaja[1], Z. Tomić[1], S. Stanković[2], T. Petrović[3], Z. Bijelić[1], V. Mandić[1], A. Obradović[2]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia
[3]Institute of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Serbia
Corresponding author: vesnakrnjaja.izs@gmail.com
Original scientific paper

Abstract: In this paper, the incidence of *Fusarium*-infected grain of winter wheat and the content of mycotoxin deoxynivalenol (DON) was studied in two Serbian cultivars Simonida and NS40S, both harvested in 2014. The level of *Fusarium* contamination of wheat grain was determined using phytopathological techniques based on the standard methodology while DON was detected by enzyme-linked immuno-sorbent assay (ELISA).

The incidence of *Fusarium*-infected grain ranged from 12 to 19% for Simonida and NS40S, respectively. *Fusarium graminearum*, as well-known producer of DON mycotoxin, was identified among *Fusarium* species. In addition, *Alternaria spp.* was isolated in high percentage, with an average incidence of 53% (Simonida) to 63% (NS40S).The average content of DON ranged from 424 μg kg^{-1} to 1101 μg kg^{-1} for Simonida and NS40S cultivars, respectively. Statistically insignificant negative correlation ($r = -0.18$) was determined between *Fusarium*-infected grain and DON in the cultivar Simonida and statistically insignificant positive correlation ($r = 0.11$) in the cultivar NS40S.

The mean levels of DON in studied wheat samples of both tested cultivars were not higher than the maximum permitted limit (1250 μg kg^{-1}) although the level of *Fusarium*-infected grain of both cultivars was relatively high. These results indicate that both wheat cultivars are susceptible to *Fusarium* infection and DON mycotoxin production in agro-ecological conditions of Serbia, but the cultivar NS40S being more susceptible compared to cultivar Simonida. In view of all stated above, regular health check of grains and developing strategies for integrated monitoring of incidence of Fusarium head blight are necessary preventive measures in protection of winter wheat.

Key words: *Fusarium* spp., deoxynivalenol, winter wheat

Introduction

Wheat is one of the most important cereal crop grown in Serbia, on approximately 500,000 ha, with average yield of 3,700 kg/ha *(Statistical Yearbook of Serbia, 2012)*. Besides maize, it is the main crop for human consumption, commonly used in the production of bread. It can also be applied in livestock nutrition, as an integral part of the feed mixture, while the straw is used as bedding in barns and stables.

The major fungal wheat disease is Fusarium head blight (FHB) *(Santos et al., 2013)*. In agro-climatic condition in Serbia, *Fusarium graminearum* has been mostly isolated from *Fusarium*-infected grains *(Lević et al., 2008; Krnjaja et al., 2011)*. There are different mycotoxins produced by this species, of which the major contaminant of cereal grains is deoxynivalenol (DON) *(Nakajima, 2007a; Stanković et al., 2012)*.

DON belongs to a larger group of trichothechenes, the type B-trichothecene, with toxic effects on animals and human health. In livestock production, DON is the main cause of reproductive disorders in pigs *(Biagi, 2009)*. In cattle, DON causes reduced food intake and lower milk production *(Trenholm et al., 1985)*. DON concentrations significantly increase in *Fusarium*-damaged grain *(Wegulo, 2012)*.

During the growing seasons in the field, the main factor responsible for FHB development and DON contamination is highly associated with weather conditions during the period of anthesis as the most susceptible growth stage for *Fusarium* infection. Intense rainfall during the anthesis disperses *Fusarium* inoculums from crop residues and promotes FHB infection *(Landschoot et al., 2012)*. Likewise, crop residues and infested debris are reported such as the major sources of the primary inoculum for *Fusarium* spp. involved in FHB epidemics *(Miedaner et al., 2008)*. According to *Blandino et al. (2012)* FHB infection and DON contamination of wheat grains caused by different factors, at first place climatic conditions, but also agronomic factors including previous crop residue management, cultivar susceptibility and fungicide applications. Similar to that, *Eiblmeier and von Gleissenthall (2007)* have stated that weather conditions at flowering, preceding crop, no or minimal tillage, susceptible cultivar, strobilurin as foliar fungicide (EC 31 – EC 59) and late harvest represent risk factors for increasing levels of DON in wheat grains. Therefore an integrated approach to the disease is appropriate to reduce the risk of high DON values in wheat grains.

Taking into consideration the importance of the harmful effect of *Fusarium* species and deoxinivalenol in wheat grains, the presence *Fusarium* spp. and deoxynivalenol (DON) in grains of two Serbian winter wheat cultivars, was analysed.

Materials and Methods

A total of 40 grain samples of two Serbian wheat cultivars, 20 samples of cultivar Simonida and 20 samples of cultivar NS40S were analysed in mycological and mycotoxicological tests. Grain samples were collected during harvest in 2014, from the crops production of the Institute for Animal Husbandry, Belgrade, Serbia. The tested wheat crops were sown in October 2013 in the field where maize was grown previously. During the growing season, the same basic cultural practices and crop protection measures were carried out for wheat crops of both cultivars, fertilization in late February was performed with ammonium nitrate fertilizer, crop protection measures against weeds, insects and pathogenic fungi were carried out at the wheat tillering stage, and treatment with fungicides against FHB was performed at the beginning of flowering. Samples of wheat grains were collected according to the method of the *European Commission (2006a),* and the moisture content of the grain was determined by moisture analyzer (OHAUS MB35, USA).

For the mycological analysis, grains were disinfected in 1% sodium hypochlorite (NaOCl) for 2-3 minutes, and rinsed twice in distilled water. After drying on filter paper, 100 of grains, of each sample, were distributed in Petri dishes with the nutrient medium (10 grains per Petri dish). Plates were incubated for 14 days at 20°C with alternating light and darkness. Two percent (2%) water-agar (WA) was used as nutrient medium, with NaCl (18g NaCl per 1 litre of medium) added to prevent the grain germination. Identification of colonies of fungi that have developed around the grains of wheat was done based on microscopic examination of mycelia and spores, as reported by *Burgess et al. (1994)* and *Watanabe (1994).* The incidence of fungal species were calculated according to *Lević et al. (2012)*: *I (%) = [Number of kernels in one sample in which a species occurred] / [Total number of kernels in the same sample] x 100.*

For the mycotoxicological analysis, the wheat grain samples were ground to a fine powder with an analytical mill (IKA A11, Germany). All the samples were kept at 4ºC in the refrigerator before further analysis. Five grams of powder was mixed with 1 g of NaCl and homogenized in 25 ml of 70% (v/v) methanol in a 250 ml Erlenmeyer flask on the orbital shaker (GFL 3015, Germany). Homogenate was filtered through a Whatman filter paper 1. The level of DON was detected using the competitive ELISA method according to the manufacturer's instructions Celer Tecna ® ELISA kits. Absorbance was determined at a wavelength of 450 nm on an ELISA plate reader spectrophotometer (Biotek EL x 800TM, USA). The limit of detection (LOD) was 40 μg kg^{-1} and the limit of quantification (LOQ) was 125 μg kg^{-1} for wheat for DON. The mean recovery turned to be 104±12%.

The correlation between individual values for moisture content, the incidence of *Fusarium* spp. and DON concentration was determined using the Pearson correlation coefficient.

Results

According to the data of Republic Hydro-meteorological Service of Serbia, for 2014 for Belgrade area, from 1 to 31 May abundant rainfall (278.5 mm) was recorded, when wheat was in the pheno stage of flowering. These were very suitable climatic conditions for the development of *Fusarium* infection on spike wheat.

The average moisture content of the tested samples of wheat was 9.96% (ranged from 9.30 to 10.29%) for the cultivar Simonida and 10.38% (ranged from 9.56 to 11.16%) for the cultivar NS40S. According to mycological analysis the species from the genera *Alternaria* and *Fusarium* were determined with highest incidence. Based on morphological characteristics, *F. graminearum* was identified as only producer of DON mycotoxin. In case of Simonida cultivar, in the examined samples, incidence of *F. graminearum* was 12% (ranged from 7-19%) wheras in the cultivar NS40S it was 19% (ranged from 5-35%). The incidence of *Alternaria* spp. was 53% (ranged from 38-65%) in the cultivar Simonida, and 63% (range 46-72%) in the cultivar NS40S (Table 1).

Table 1. Incidence of *Fusarium graminearum* and *Alternaria* spp. in tested grain samples of two Serbian wheat cultivars

Fungal species	Wheat cultivar			
	Simonida		NS40S	
	Incidence (%)			
	Range	Average	Range	Average
Alternaria spp.	38-65	53	46-72	63
Fusarium graminearum	7-19	12	5-35	19

The presence of DON was detected in 100% of tested samples of both cultivars. The average concentration of DON was 424 μg kg^{-1} (ranged from 175 – 610 μg kg^{-1}) in the cultivar Simonida, and 1101 μg kg^{-1} (ranged from 214-1440 μg kg^{-1}) in the cultivar NS40S (Table 2). The average concentrations of DON in unprocessed wheat grain of both tested cultivars were not above the maximum permitted limit (1250 μg kg^{-1}) adopted by the *European Commission (2006b).*

Table 2. Level of DON in tested grain samples of two Serbian wheat cultivars

Mycotoxin	DON	
Wheat cultivar	Simonida	NS40S
Sample size[a]	20/20	20/20
Incidence %	100	100
Range (μg kg^{-1})	175-610	214-1440
Mean[b] (μg kg^{-1})	424	1101

[a] Number of positive samples/Number of total samples; [b] Mean concentration in positive samples

Statistically insignificant negative correlation (r = - 0.18) was determined between the incidence of *F. graminearum* and concentrations of DON in the cultivar Simonida, and statistically insignificant positive correlation (r = 0.11) in the cultivar NS40S. The correlation between moisture content and incidence of *F. graminearum* evident in both cultivars was statistically insignificant negative, while between moisture content and concentration of DON statistically insignificant positive correlation was found in both tested cultivars.

Discussion

In this study, a high incidence of *F. graminearum* was established in the investigated samples of wheat grains of both studied cultivars, although, cultivar NS40S had higher average incidence of *F. graminearum* (19%) compared to the cultivar Simonida (12%). Similarly, the average concentration of DON in Simonida was lower (424 μg kg^{-1}) in relation to the cultivar NS40S (1101 μg kg^{-1}). These results are similar to those reported by *Krnjaja et al. (2014)* where the average incidence of *F. graminearum* was 14% and the average concentration of DON was 478 μg kg^{-1} in the 19 tested wheat samples of the Serbian cultivar Takovčanka, collected during the harvest 2013. It should be noted that in all these trials, grains sampled originated from the wheat crop treated with fungicide against FHB at the flowering stage of development. Based on earlier research *of Krnjaja et al. (2011a, b)*, the average incidence of *F. graminearum* was higher than 80% in wheat grain samples originating from crops that were not treated with fungicide at the flowering stage of development. The average concentration of mycotoxin DON ranged from 214 to 490 μg kg^{-1}*(Krnjaja et al., 2011a, b)*. These results pointed out that the agro-ecological and agro-climatic conditions in Serbia are very suitable for the occurrence of FHB and production of DON mycotoxin in wheat grain. Furthermore, maize as the previous crop and important host species for *Fusarium* spp. provided more inoculum especially in favourable weather conditions such as heavy precipitation during anthesis of wheat in 2014. In our study, differences were found between the cultivars in terms of the incidence of *F. graminearum* and the DON content. This indicated that the use of less susceptible cultivars to FHB can be an important factor in reducing the occurrence of harmful contaminants in wheat grain.

According to *Mesterházy et al. (1999)* resistance of cultivars has also an impact on the contents of DON. In most resistant cultivars, a low average level of DON was detected, and in some growing seasons, DON was not detected at all. In addition, great differences in the content of DON in the same cultivar of wheat have been also established, that involved a large diversity of pathogenic populations in the field *(Mesterházy et al., 1999)*. According to recent research of *Mesterházy et al. (2002)*, it was concluded that the level of resistance of given

cultivar was more important in accumulation of DON than the aggressiveness of *Fusarium* isolates. This is confirmed by our research in which the differences among the cultivars in terms of accumulation of DON were determined, although the incidence of *F. graminearum* was high in both wheat cultivars. *Blandino et al. (2012)* reported that the main factors affecting the formation of DON in wheat grain were; susceptibility of a wheat cultivar, the preceding crop (especially maize and sorghum), soil tillage, fungicide application at anthesis of wheat. Contrary to the above, *Müller et al. (2010)* pointed out that the monitoring of FHB and DON content requires good knowledge of agricultural factors, primarily crop rotations and tillage practice, then climatic conditions especially annual precipitation and topographic factors (relief position, topographic wetness index TWI). In the research of these authors, the susceptibility ranking of wheat cultivars to *Fusarium* infection had no significant influence on DON accumulation.

According to data from other countries with similar geographical and climatic conditions, *Mankevičiene et al. (2007)* have established the incidence of DON in 62.5% to 100% of cereal samples from harvests 2004 and 2005 in Lithuania, with concentrations ranged up to 1121μg kg^{-1}. The high concentrations of DON were highly associated with abundant rainfall in both investigated years. Likewise, *Pleadin et al. (2012)* revealed the high mean concentrations of DON (up to 1454μg kg^{-1}) in 103 feed samples from three regions of Croatia.

Conclusion

It can be concluded that both tested cultivars were susceptible to FHB, although, NS40S cultivar, showed higher incidence of *F. graminearum* and higher DON content compared to the cultivar Simonida. Although the average DON levels did not exceed the maximum permitted limit, it is necessary to develop and implement the methods for monitoring the risk of harmful contaminants in wheat grain. Therefore, it is essential to conclude that the individual methods such as the application of less sensitive or tolerant cultivars in the strategy of control of FHB and mycotoxins in wheat grain, have lower effect in preventing the risk of harmful contaminants in the food chain, compared to a combination of several measures of crop protection such as crop rotation, tillage practice and fungicide application at anthesis of wheat, especially under favourable climatic conditions, existing in Serbia.

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia within projects TR-31023, TR-31053 and TR-46010.

Fusarium infekcija i deoksinivalenol kontaminacija ozime pšenice

V. Krnjaja, Z. Tomić, S. Stanković, T. Petrović, Z. Bijelić, V. Mandić, A. Obradović

Rezime

U radu je proučavana incidenca *Fusarium*-zaraženih zrna ozime pšenice iz žetve 2014. godine kod dve domaće komercijalne sorte Simonida i NS40S, kao i sadržaj mikotoksina deoksinivalenola (DON). Nivo fuzariozne kontaminacije zrna pšenice određen je primenom fitopatoloških testova standardne metodologije a DON je detektovan primenom imunoadsorpcione enzimske metode (ELISA).

Incidenca *Fusarium*-zaraženih zrna bila je u proseku za sve ispitivane uzorke od 12% (Simonida) do 19% (NS40S). Od *Fusarium* vrsta identifikovana je jedino *Fusarium graminearum*, kao dobro poznati producent DON mikotoksina. Pored ove gljivične vrste, u visokom procentu izolovana je *Alternaria* spp. sa prosečnom incidencom od 53% (Simonida) do 63% (NS40S). U ispitivanim uzorcima pšenice prosečan sadržaj DON bio je od 424 μg kg^{-1} (Simonida) do 1101 μg kg^{-1} (NS40S). Između *Fusarium*-zaraženih zrna i DON utvrđena je statistički neznačajna negativna korelacija ($r = – 0.18$) kod sorte Simonida i statistički neznačajna pozitivna korelacija ($r = 0.11$) kod sorte NS40S.

Prosečne koncentracije DON u ispitivanim uzorcima pšenice kod obe ispitivane sorte nisu bile iznad maksimalno dozvoljenog limita iako je nivo fuzariozne kontaminacije zrna obe ispitivane sorte bio visok. Ovi rezultati ukazuju da su obe ispitivane sorte pšenice osetljive prema fuzarioznoj infekciji i produkciji DON mikotoksina u agroekološkim uslovima Srbije, s tim što je sorta NS40S osetljivija u odnosu na sortu Simonida. Zbog svega navedenog, redovna zdravstvena kontrola zrna i razvijanje strategije integralnog monitoringa fuzarioze klasa neophodne su preventivne mere borbe u zaštiti pšenice.

References

BIAGI G. (2009): Dietary supplements for the reduction of mycotoxin intestinal absorption in pigs. Biotechnology in Animal Husbandry, 25, 5-6, 539-546.

BLANDINO M., HAIDUKOWSKI M., PASCALE M., PLIZZARI L., SCUDELLARI D., REYNERI A. (2012): Integrated strategies for the control of Fusarium head blight and deoxynivalenol contamination in winter wheat. Field Crop Res., 133, 139-149.

BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory Manual for *Fusarium* Research. Third

edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney, pp. 133.
EIBLMEIER P., VON GLEISSENTHALL J.L. (2007): Risk evaluation of deoxynivalenol levels in Bavarian wheat from survey data. Journal of Plant Disease and Protection, 114, 2, 69-75.
EUROPEAN COMMISSION (2006a): Commision regulation (EC) No. 401/2006. Official Journal of the European Union, L70, 12.
EUROPEAN COMMISSION (2006b): Commission regulation 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official J. Eur. Union L 364, 5-24.
KRNJAJA V.S., LEVIĆ J.T., STANKOVIĆ S.Ž., STEPANIĆ A.M. (2011a): *Fusarium* species and their mycotoxins in wheat grain. Proc. Nat. Sci, Matica Srpska Novi Sad, 120, 41-48.
KRNJAJA V., LEVIĆ J., STANKOVIĆ S., PETROVIĆ T., MANDIĆ V., TOMIĆ Z., OBRADOVIĆ A. (2014): Presence of deoxynivalenol in winter wheat treated with fungicides. Biotechnology in Animal Husbandry, 30, 1, 167-173.
KRNJAJA V.S., STANKOVIĆ S.Ž., LEVIĆ J.T., (2011b): The presence of toxigenic *Fusarium* species and fusariotoxins deoxynivalenol and zearalenone in winter wheat. Biotechnology in Animal Husbandry, 27, 1, 63-73.
LANDSCHOOT S., WAEGEMAN W., VANDEPITTE J., BAETENS J.M., BAETS B.DE., HAESAERT G. (2012): An empirical analysis of explanatory variables affecting Fusarium head blight infection and deoxynivalenol content in wheat. J. Plant Pathol., 94, 1, 135-147.
LEVIĆ J., STANKOVIĆ S., KRNJAJA V., BOČAROV-STANČIĆ A., IVANOVIĆ D. (2012): Distribution frequency and incidence of seed-borne pathogens of some cereals and industrial crops in Serbia. Pesticide and Phytomedicine, 27, 1, 33-40.
LEVIĆ J., STANKOVIĆ S., KRNJAJA V., KOVAČEVIĆ T., TANČIĆ S. (2008): Fusarium head blight and grain yield losses of Serbian wheat. Cereal Research Communications 26, Suppl. B, 513-514.
MANKEVIČIENE A., BUTKUTĖ B., DABKEVIČIUS Z., SUPRONIENĖ S. (2007): *Fusarium* mycotoxins in Lithuanian cereals from the 2004-2005 harvests. Ann. Agric. Environ. Med., 14,103-107.
MESTERHÁZY Á. (2002): Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to Fusarium head blight. European Journal of Plant Pathology, 108, 675-684.
MESTERHÁZY Á., BARTÓK T., MIROCHA C.G., KOMORÓCZY R. (1999): Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breeding, 118, 97-110.
MIEDANER T., CUMAGUN C., CHAKRABORTY S. (2008): Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*. Journal of Phytopathology, 156, 129-139.

MÜLLER M.E.H., BRENNING A., VERCH G., KOSZINSKI S., SOMMER M. (2010): Multifactorial spatial analysis of mycotoxin contamination of winter wheat at the field and landscape scale. Agriculture, Ecosystems and Environment, 139, 245-254.
NAKAJIMA T. (2007): Making evidence-based good agricultural practice for the reduction of mycotoxin contamination in cereals. Food and Fertilizer Technology Center, Annual Report, 05-17, 111-120.
PLEADIN J., PERŠI N., VULIĆ A., ZADRAVEC M. (2012): Survey of mycotoxin feed contamination in Croatia. Biotechnology in Animal Husbandry, 28, 2, 167-177.
SANTOS J.S., SOUZA T.M., ONO E.Y.S., HASHIMOTO E.H., BASSOI M.C., MIRANDA M.Z., ITANO E.N., KAWAMURA O., HIROOKA E.Y. (2013): Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products. Food Chem., 138, 90-95.
STANKOVIĆ S., LEVIĆ J., IVANOVIĆ D., KRNJAJA V., STANKOVIĆ G., TANČIĆ S. (2012): Fumonisin B1 and its co-occurrence with other fusariotoxins in naturally-contaminated wheat grain. Food Control 23, 384-388.
STATISTICAL YEARBOOK OF SERBIA (2012): Statistical Office of the Republic of Serbia, Belgrade, pp. 410.
TRENHOLM H.L., THOMPSON B.K., HARTIN K E., GREENHALGH R., McALLISTER A.J. (1985): Ingestion of vomitoxin deoxynivalenol-contaminated wheat by nonlactating dairy cows. J. Dairy Sci., 68, 4, 1000-1005.
WATANABE T. (1994): Pictorial atlas of soil and seed fungi. In: Morphologies of cultured fungi and key to species. Lewis Publishers, Boca Raton, Boston, London, Washington D.C. pp. 410.
WEGULO S.N. (2012): Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins, 4, 1157-1180.

Received 9 January 2015; accepted for publication 11 March 2015