SHORT COMMUNICATION

Natural occurrence of deoxynivalenol in kernels of wheat grown in Slovakia during 2004–2008

VALÉRIA ŠUDYOVÁ – SVETLANA ŠLIKOVÁ – EDITA GREGOVÁ

Summary
At nine locations in 2004–2008, the incidence of deoxynivalenol (DON) in grains of wheat grown in Slovakia was studied, with a focus on samples that are important for production of cereal products. Altogether 184 wheat samples collected directly from growers were analysed for DON contents using the ELISA method. Year and location had a significant effect on DON concentration. There was a positive correlation between DON content and rainfall at given locations. The limit of 1.25 mg·kg^{-1} imposed by the European Union (EU) for DON content was exceeded in 10.3% of the studied samples. The results show the tendency of increased toxin contamination of wheat grown in locations with higher rainfall during and after wheat flowering period and it also revealed the locations with frequent incidence of samples exceeding the EU limit in Slovakia.

Keywords
winter wheat; deoxynivalenol; location; year

The mycotoxin deoxynivalenol (DON) has a toxic effect on animals and its presence in feed is a cause of reduced feed intake, considerable loss of body weight and vomiting in exposed animals [1, 2]. The toxic effects of deoxynivalenol on animal and plant cells, as well as a high stability of trichothecenes during cooking, normal food processing, autoclaving, and also at neutral and acidic pH, were reported by ROCHA et al. [3]. The cytotoxic effect of DON on human liver cells is reported in the study of KÖNIGS et al. [4]. With regard to the general toxicity of DON, the Scientific Committee on Food established in 2005 the tolerable daily intake value (TDI) for deoxynivalenol (DON) of 1 μg per 1 kg of body weight per day. There are limits to the amount of *Fusarium* mycotoxins acceptable in unprocessed grain for food production in the European Union (EU) [5]. The studies have revealed that DON is the main representative of *Fusarium* toxic secondary metabolites and its incidence was confirmed in various wheat samples and products [6, 7]. The study of KUSHIRO [8] documented that DON is reduced step by step during cleaning, milling and cooking, but is not completely eliminated from final products. During baking or heating DON is partially degraded to DON-related chemicals (3-keto DON and DON-3-glucoside), whose toxicological effects have not yet been well studied. The incidence and quantity of DON toxin in wheat grain, according to KOCH et al. [9], depends on rainfall during flowering, forecrop, variety susceptibility to disease and the system of growing.

The present paper reports on results of the study of natural incidence of deoxynivalenol in the samples of wheat for food use. Samples were obtained immediately after harvest of wheat in nine locations of Slovakia during five years of cultivation. Locations were chosen on the basis of agroclimatic conditions that gave the presumption of different amounts of rainfall during the flowering and after wheat flowering in June.

MATERIALS AND METHODS

In the growing season between 2004 and 2008, mature wheat grain were collected for the monitoring of DON contents. Approximately 500 g of kernel samples were recovered from the same

Valéria Šudyová, Svetlana Šliková, Edita Gregová, Plant Production Research Centre Piešťany, Bratislavská cesta 122, SK – 921 68 Piešťany, Slovakia.

Correspondence author:
Svetlana Šliková, e-mail: slikova@vurv.sk

farmers and from the same locality every year. The collections of samples were taken from nine locations of Slovakia: Abrahám, Želiezovce, Veľký Meder, Veľké Ripňany, Haniska, Turčiansky Ďur, Vranov nad Topľou, Malý Šariš and Spišské Vlachy.

A commercial ELISA kit was used to determine the DON concentration in wheat samples (Ridascreen Fast DON; R-Biopharm, Darmstadt, Germany). The kernel samples were ground (Ultra Centrifugal Mill, type ZK 100; Retsch, Haan, Germany), subsequently 100 ml of distilled water was added to 5 g of each sample and the mixture was filtered. A volume of 50 μl of the filtrate was used for analysis. The absorbances in wells were determined spectrophotometrically at 450 nm (MRX II; Dynex Technologies, Chantilly, Virginia, USA). DON concentrations were calculated in mg·kg^{-1} by Revelation Version 4.25 (Dynex Technologies).

The data were calculated by analysis of variance (ANOVA) using SPSS software (SPSS, Chicago, Illinois, USA) and, for the assessment of the relationship between the traits, Pearson's correlation analysis was applied using Microsoft Excel 97 SR-2 software (Microsoft Corporation, Redmond, Washington, USA).

RESULTS AND DISCUSSION

ANOVA of DON contents in 184 wheat samples collected in the years 2004–2008 from nine locations showed significant differences between the years and locations (Tab. 1). The results in Tab. 2 show that the highest average accumulation of DON in the samples was in 2006 and the lowest in 2005. The average rainfall was the highest in 2006 and the lowest in 2005 in June, when wheat was, according to the phenological observations,

Tab. 1. ANOVA for deoxynivalenol content in wheat samples collected during 2004–2008 from 9 locations of Slovakia.

Parameter	Source of variation	Degree of freedom	Mean square	*F*-value	*P*-value
DON [mg·kg^{-1}]	Year	4	1.752	2.640	0.036
	Locality	8	4.778	7.201	0.000
	Year * Locality	32	0.883	1.330	0.133
	Error	139	0.664		

* – interaction between two factors.

Tab. 2. The content of DON in wheat samples collected in 2004–2008 from 9 locations of Slovakia.

Year and locality	Number of samples analysed	DON content of samples [mg·kg^{-1}]		Percent of samples > 1.2 mg·kg^{-1} [%]
		Range	Mean	
2004	44	0.2–7.2	0.75^{ab}	2.7
2005	37	0.2–2.2	0.39^{a}	0.5
2006	30	0.2–1.7	0.82^{b}	3.3
2007	36	0.2–3.8	0.58^{ab}	2.7
2008	37	0.2–8.7	0.55^{ab}	1.0
Veľký Meder	17	0.2–0.8	0.29^{a}	0.0
Haniska	27	0.2–0.9	0.38^{a}	0.0
Abrahám	17	0.2–1.3	0.41^{a}	0.5
Želiezovce	20	0.2–1.5	0.43^{a}	1.6
Malý Šariš	26	0.2–1.6	0.46^{a}	0.5
Veľké Ripňany	16	0.2–1.9	0.46 ^{a}	0.5
Spišské Vlachy	23	0.2–2.2	0.60^{a}	1.6
Vranov nad Topľou	19	0.2–3.0	0.69^{a}	1.1
Turčiansky Ďur	19	0.4–8.7	1.89^{b}	4.3

a, b – by Duncan test.

Tab. 3. Rainfall in June in 2004–2008 from 9 locations of Slovakia.

Locality	2004	2005	2006	2007	2008	Mean
	[mm]					
Abrahám	98	53	98	81	101	86.2
Haniska	98	62	139	67	71	87.4
Malý Šariš	86	105	111	69	55	85.2
Spišské Vlachy	136	118	248	62	44	121.6
Veľký Meder	87	56	74	82	94	78.6
Veľké Ripňany	133	42	65	59	55	70.8
Vranov nad Topľou	93	85	121	75	126	100.0
Želiezovce	119	37	107	75	97	87.0
Turčiansky Ďur	122	45	67	127	144	101.0
Mean	108.0	67.0	114.4	77.4	87.4	90.9

in the flowering stage or after flowering (Tab. 3). MESTERHÁZY [10] pointed out that there is a direct relationship between the amount of DON accumulated in wheat grains and the rainfall during and after wheat flowering. According to COWGER [11], extended post-flowering moisture may have a significant enhancing effect on DON in wheat grains as well. The average accumulation of DON in 2004 was higher by 22.9% and in 2006 by 34.4% in comparison to the total average accumulation of DON (0.61 mg·kg^{-1}). In other years, accumulation of DON in the samples was lower than the total average accumulation by 36.0% (year 2005), 4.9% (year 2007) and in the year 2008 by 9.8% (Tab. 2). Similar high differences in DON contents between the years were found in wheat samples which originated from 8 locations in Romania [12]. Significant differences in contamination with DON between the years were also found in samples collected from the mills and the retail market in Denmark during the period 1998–2003. In the year with the highest rainfall around flowering time, the samples were contaminated with this mycotoxin the most [13]. In our study, the highest average accumulation of DON was in the locality Turčiansky Ďur and the lowest in Veľký Meder (Tab. 2). The total average accumulation of DON (0.61 mg·kg^{-1}) was three times lower than in the locality Turčiansky Ďur and 6.5 times higher than in the locality Veľký Meder. The number of average rainfall in June in Turčiansky Ďur was 1.3 times higher than in Veľký Meder (Tab. 2). Close correlation was found between the quantity of rainfall measured at locations in the month of June and the average contamination of samples from the locality (Fig. 1).

According to FRANZ [14], the level of DON content in wheat samples increased with a higher average temperature, increased precipitation, and higher relative humidity, but decreased with increased number of hours with the temperature above 25 °C. In our study, the highest range of contamination of the samples by the mycotoxin was found in 2008 and the lowest in 2006 (Tab. 2). In other years, the high range of samples contamination was related to the rare incidence of samples with a high DON content in certain locations (Tab. 2).

AUDENAERT et al. [15] analysed samples of various wheat varieties from seven different locations and found out significant differences in DON content among the samples in each locality. The strong regional effect on DON content could be

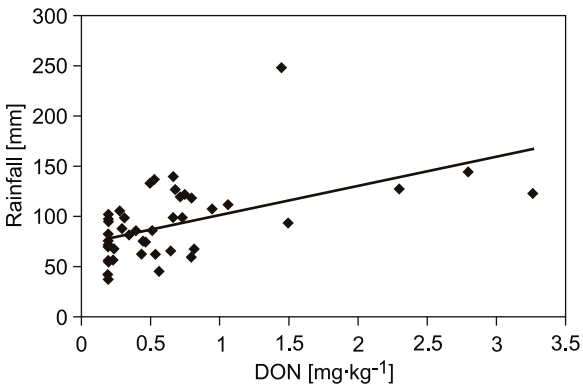

Fig. 1. Relationship between DON content and the sum of rainfall in June from nine locations in 2004–2008 by Pearson's correlation analysis of coeficient (*r*).

seen in winter wheat samples, collected from the fields in the Netherlands with the aim to develop predictive models for DON content in this crop [14]. According to SCHAAFSMA and HOOKER [16], weather conditions affect occurrence of the mycotoxin DON in wheat grain at 48%, variety 27% and growing conditions 14–28%, with 4 to 7 days before flowering and 3 to 10 days after flowering being the periods most associated with DON contamination of samples.

The limit for DON content imposed by the European Union (1.25 $mg \cdot kg^{-1}$) was exceeded in 10.3% (19 samples) of the total number of analysed samples. The average accumulation of DON in the samples, which exceeded the limit of EU, was 2.4 $mg \cdot kg^{-1}$. The percentage of occurrence of these samples between years varied (Tab. 2). The highest frequency of occurrence of samples with excess content of DON from nine locations was in year 2006 and the lowest was in 2005, when the average yearly rainfall from 9 locations was the lowest (Tab. 3). The highest occurrence of samples exceeding the permissible limit of EU was in the locality Turčiansky Dur. No such sample was found in Veľký Meder and Haniska locations within the studied years (Tab. 2). The incidence of samples exceeding the permissible limit for DON content of wheat grown in different locations and years was recorded in many works [12, 17].

CONCLUSIONS

Analysis of DON content in grain of wheat grown in the territory of Slovakia in nine locations during five years showed highly significant differences in mycotoxin contamination of grains. Confirmed was the relationship between the amount of rainfall during and after flowering of wheat and mycotoxin contamination of grains. The percentage of samples that exceeded the permissible legal limit for the DON content in wheat samples fluctuated between the years. In the areas with relatively frequent occurrence of samples with DON content above the limit, it is important for a producer to pay attention to many factors that affect the incidence of *Fusarium* species in order to reduce the risk of mycotoxin contamination. This study documents the potential for the production of DON in wheat grown in Slovakia and the need to monitor the incidence of DON in locations with frequent occurrence of contaminated samples to ensure the safety of cereal products.

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