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PRESENCE OF DEOXYNIVALENOL IN SMALL-GRAIN SAMPLES FROM 2009/10 HARVEST SEASON

ABSTRACT: *Fusarium* head blight (FHB) is present in all growing regions of small grains and causes significant losses in yield and grain quality. In our environmental conditions, dominant species is *Fusarium graminearum* Group 2. During 2009/10 there was a significant *Fusarium* infestation on wheat, barley and triticale. The aim of this study was to examine the contents of deoxynivalenol (DON) in cereal samples taken after 2009/10 harvest season. We analyzed 22 NS varieties of small grains from Rimski Šančevi, including 16 varieties of winter wheat, one facultative wheat variety, four varieties of winter barley and one variety of triticale.

Analytical methods based on clean-up by solid-phase extraction (SPE) columns and detection by liquid chromatography were used. Fifteen out the 22 analyzed samples were positive for the presence of DON at a mean level of 0.537 mg/kg. The highest concentration was 1.952 mg/kg. These findings were in correlation with percentage of the *Fusarium* damaged kernels.

KEY WORDS: barley, deoxynivalenol, triticale, wheat

INTRODUCTION

Fusarium head blight (FHB) is the most destructive disease of small grains which causes significant losses in yield and grain quality. In our environmental conditions the dominant species is *Fusarium graminearum* Group 2 (B a g i, 1999). During the years with higher amounts of rainfall, the frequency of *F. culmorum* is greater. Besides these two species, *F. avenaceum* is also present in our fields, but it has much lower pathogenicity.

Other *Fusarium* species do not cause typical symptoms, but can be important from the aspect of production of mycotoxins in grains (S t o j a n o v i ć et al., 1998), which have harmful effects on human and animal health. Among

toxins produced by the pathogens of the genus *Fusarium*, the most frequent and the most important ones in a great number of agricultural products are deoxynivalenol (DON) and zearalenone (ZEA) toxins (JECFA, 2001). Deoxynivalenol is a member of the trichothecene family of mycotoxins. It is among the least toxic of the trichothecenes, but it is the most frequently detected one throughout the world. Its occurrence is considered to be an indicator of possible presence of other, more toxic trichothecenes (L o m b a e r t, 2002). Consumption of contaminated feeds by livestock has been associated with a variety of adverse health effects, including feed refusal, reduced weight gain, diarrhoea and emesis (K r s k a et al. 2001; K u i p e r-G o o d m a n, 2002).

The Commission of the European Communities (Commission Regulation 1881/2006) established the following tolerance values for DON in cereals and cereal-based products: unprocessed cereals other than durum wheat, oats and maize (1250 µg/kg), unprocessed durum wheat and oats (1750 µg/kg), unprocessed maize (1750 µg/kg), cereal flour, including maize flour, maize grits and maize meal (750 µg/kg), bread, pastries, biscuits, cereal snacks and breakfast cereals (500 µg/kg), pasta (dry, 750 µg/kg), and processed cereal-based food for infants and young children and baby food (200 µg/kg). The maximum permitted level of DON has been set in our country only in feed (Official Gazzette 2010) and in complete feeding stuffs for pigs, which is 500 µg/kg.

The aim of this study was to estimate the contents of DON in cereal samples (wheat, barley and triticale) taken after 2009/10 harvest season.

MATERIALS AND METHODS

Samples

Sampling of 22 NS varieties of cereals, including 16 varieties of winter wheat, one facultative wheat variety, four varieties of winter barley and one variety of triticale was performed. Immediately after sampling, 500 g of each sample were prepared by grinding in a laboratory mill, and the sample was homogenized by mixing. Samples prepared in such a way were packed in plastic bags and stored in a freezer at -20^0C until the analysis was carried out. Prior to each analysis, the samples were allowed to reach room temperature.

Extraction and clean-up

Mycosep 225 column (Romer Labs, USA) was used for purification. Amount of 25.0 g of the sample was extracted with 100 ml of acetonitrile–water (84:16, v/v) and shaken on an Ultra Turrax (IKA, Germany) for 3 min. After filtration, through Advantec filter paper, 3.0 ml of the extract were applied to the Mycosep 225 column. The cleaned-up extract was evaporated to dryness, dissolved in 3 ml of ethyl acetate and quantitatively transferred to an

evaporation vessel by triple washing with 1.5 ml ethyl acetate. The eluate was evaporated up to the dryness.

Liquid chromatographic analysis

The equipment consisted of an LC system – Liquid Chromatograph Agilent 1200 series (Agilent Technologies, USA) with a DAD detector (Agilent Technologies, USA) and a column Hypersil ODS (100 x 4.6 mm i.d., particle size 5 µm, Agilent Technologies, USA). LC analysis of DON was performed after evaporation, the residue was redissolved in 300 µl methanol, and a 15 µl aliquot of the solution was injected into the LC system. A mobile phase consisting of a mixture of acetonitrile–water (14:86, v/v) was used at 0.8 ml/min. UV detection was performed at 220 nm. Calibration curves used for quantitative determination were constructed on the basis of the area under DON chromatographic peaks, using working standard solutions. The detection limit measured as signal-to-noise ratio (3:1) was 0.045 µg/ml for DON which corresponds to 0.018 µg/g DON in wheat. The limit of quantification was 0.15µg/ml for DON which corresponds to 0.06 µg/g DON in wheat, and it is significantly lower than the recommended maximum permitted level.

RESULTS

Presence of DON in 22 samples of small-grain cereals is shown in Table 1.

Tab. 1 – Occurrence of deoxynivalenol in small-grain cereal samples from the 2010 harvest season

Cereal	**No. of samples**	**No. of positive samples (%)**	**Concentration in samples**	
			Average (mg/kg ± SD)	**Range (mg/kg)**
Wheat	17	14 (82.4)	0.435±0.43	0.068-1.572
Barley	4	–	–	–
Triticale	1	1 (100)	1.952	1.952
Total	22	15 (68.2)	0.537±0.57	0.068-1.952

Out of 17 analyzed wheat samples, even 14 samples (82.4%) were positive for the presence of DON. Concetration range of DON was 0.068-1.572 mg/kg, with average content of 0.435 mg/kg. None of the 4 analyzed barley samples was positive on the presence of DON. The highest concentration of DON was found in a single sample of analyzed triticale, and it was 1.952 mg/kg. The overall presence of DON in all analyzed samples was as high as 68.2%, with the average DON content being 0.537 mg/kg.

Content of DON in all varieties of small-grain cereals is shown in Table 2.

Tab. 2. – Content of deoxynivalenol in small-grain varieties

No.	Variety	Cereal	Concentration (mg/kg)
1	Nov 525	Barley	ND
2	Atlas 2010	Barley	ND
3	Nonius 2010	Barley	ND
4	Nov 565 2010	Barley	ND
5	Evropa 90 2010	Wheat	0.076
6	Rusija 2010	Wheat	0.708
7	Pesma 2010	Wheat	0.240
8	Renesansa	Wheat	ND
9	Pobeda 2010	Wheat	0.852
10	Ljiljana 2010	Wheat	0.084
11	Dragana	Wheat	0.088
12	Simonida 2010	Wheat	ND
13	Arija	Wheat	0.692
14	Angelina 2010	Wheat	0.276
15	40 S 2010	Wheat	0.532
16	Etida 2010	Wheat	0.088
17	Zvezdana 2010	Wheat	ND
18	Gordana 2010	Wheat	0.068
19	Gora 2010	Wheat	0.212
20	Vojvodina 2010	Wheat	0.608
21	Nataša 2010	Wheat	1.572
22	Odisej 2010	Triticale	1.952

DISCUSSION

Established presence of DON in wheat (82.4%) can be considered extremely high. In two samples, concentrations of DON were above the maximum level adopted by the European Commission (Table 2).

During the 2010 there were two critical periods at Rimski Šančevi for infection by fungus of the genus *Fusarium* (J e v t i ć et al., 2010a). The second period was considerably longer than the first one, and coincided with the period of flowering of most varieties. It lasted from 13-25th of May, 2010. The most critical period was from 14-18th of May, the when infections occurred on most varieties.

The percentage of *Fusarium* damaged kernels was determined in 16 wheat varieties which were included in the DON content testing. The percentage ranged from 1 to 11.5% and the calculated losses in 1000 kernel weight ranged from 1.2 to 5.7%.

In seed samples of Renesansa, Simonida i Zvezdana varieties, mycotoxin DON was not determined (Table 2). Zvezdana variety had the lowest percentage (1%) of *Fusarium* damaged kernels under natural conditions of infection (J e v t i ć et al., 2010b), which was in agreement with these results. Pobeda and Gora varieties, despite the significant percentage of infected grains, did

not have greater yield losses in 2010. Based on the observations, a certain level of tolerance to Fusarium head blight was observed. However, significant differences were observed with respect to content of DON. Nataša variety, in which concentration of DON was above the maximum level, had also a very high percentage of Fusarium head blight in field conditions. In some fields, it ranged up to 33.3% of infected spikes per m^2 (J e v t i ć et al., 2010a). In this paper, the DON content of hard (durum) wheat was not examined. However, based on previous research (unpublished data) durum wheat is very sensitive on Fusarium head blight. Therefore, we assume that the DON content in this type of wheat would have high percentage.

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ПРИСУСТВО ДЕОКСИНИВАЛЕНОЛА У УЗОРЦИМА СТРНИХ ЖИТА У ЖЕТВЕНОЈ 2009/10. ГОДИНИ

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Резиме

Патогени из рода *Fusarium* су присутни у свим рејонима гајења стрних жита и наносе значајне губитке у приносу и квалитету зрна. Фузариозу класа пшенице проузрокује већи број врста из рода *Fusarium*. У нашим условима гајења доминантна је врста *Fusarium graminearum* Група 2. У току 2009/10. године дошло је до значајне појаве фузариоза на пшеници, јечму и тритикалеу. Циљ рада је био да се испита садржај DON-а на узорцима стрних жита узетих после жетве из услова природне заразе. Анализиране су 22 новосадске сорте стрних жита из локалитета Римски Шанчеви, од чега: 16 сорти озиме пшенице, једна факултативна сорта пшенице, четири сорте озимог јечма и једна сорта тритикалеа. Узорци су узети са парцела различитих површина у зависности од значаја и распрострањености гајене сорте.

Аналитичко одређивање је засновано на пречишћавању сировог екстракта анализираних узорака помоћу тзв. Mucosep колона, а затим је садржај DON-а квантитативно одређен течном хроматографијом. Од 22 анализирана узорка стрних жита чак 15 (68,2%) је било позитивно на присуство DON-а. Још већи проценат зараженост DON-ом је утврђен када је у питању само пшеница (82,4%). Просечан садржај DON-а је износио 0,537 mg/kg а највећа концентрација је утврђена у узорку тритикалеа и износила је високих 1,952 mg/kg. Од свих узорака који су били позитивни на присуство овог микотоксина, 2 су превазилазила концентрације које су прописане од стране Европске комисије. Све ово указује на високу зараженост стрних жита са наших поља из жетве 2010. Проценат фузариозних зрна код 16 испитиваних сорти пшенице кретао се од 1 до 11,5%, а губици у маси 1000 зрна од 1,2 до 5,7%. Између јачине заразе у пољу и садржаја микотоксина DON установљена је потпуна позитивна корелација код појединих сорти. Факултативна сорта пшенице Наташа је имала високу концентрацију DON од 1,572 mg/kg, при степену заразе у пољу од 33,3% заражених класова по 1 m^2. Сорта Звездана имала је најнижи проценат заразе у пољу од 1% и код ње није детерминисано присуство микотоксина DON.