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INCIDENCE OF *FUSARIUM* MYCOTOXINS ON DIFFERENT OAT CULTIVARS UNDER NATURAL AND ARTIFICIAL INFECTION CONDITIONS

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ABSTRACT

Evaluation of the genetic variability of the oat resources regarding resistance to *Fusarium* sp. and the accumulation of mycotoxins has been achieved within the European project "Avena-genetic resources for quality in human consumption" (AVEQ-AGRI GEN RES 06.11.03.2007- 28.02.011) in two out of ten work packages.

Testing of the oat genotypes was effectuated under artificial infection conditions with different species of *Fusarium* in three locations from Europe (Romania, Czech Republic, Germany).

In the experimental field of Suceava Genebank in 2008, 11 modern cultivars originating from different parts of Europe were used as inoculated and noninoculated standards to determine the infection with *Fusarium* sp. and the presence of different mycotoxins types.

Analysis of accumulated mycotoxins in these standard cultivars was achieved using LC-MS/MS method in Eurofins laboratory Contaminants WEJ GmbH, Hamburg, Germany.

The infected panicles with *Fusarium* head blight (FHB) were visually assessed using the 1-9 scale (1 - no infection, 9 - all spikelets and panicles infected).

Quantification of the obtained results on the analysis of mycotoxins and the estimations concerning the infection on panicles and grains in inoculated and noninoculated standard genotypes from genebank experimental field were achieved through identification of *Fusarium* mycotoxins distribution, the differences between the concentrations of mycotoxins and correlations between concentrations of mycotoxins and infection with *Fusarium* head blight (FHB) and *Fusarium* damaged kernels (FDK - score 3).

Key words: *Fusarium* head blight (FHB), *Fusarium* damaged kernels (FDK), mycotoxins, standard cultivars, inoculation.

INTRODUCTION

Fusarium mycotoxins are fungus metabolites produced by different species of *Fusarium* sp. on the harvested small-grain cereals and straw which, in high concentrations, represent a risk to human and animal health.

The oat or oat products are less contaminated by mycotoxins than wheat or corn, but some recent papers group oats along with cereals with significant mycotoxin contamination potential (Veisz et al., 1997; Bottalico and Perrone, 2002; Münzing et al., 2002; Henriksen and Ellen, 2005).

Generally, oat is less susceptible to infection with *Fusarium* sp. than wheat due to the large spacing between florets (Langevin et al., 2004; Liu et al., 1997; Iacob et al., 1998), but a rather low genetic variability in modern cultivars are presumed to be detectable (Tekauz et al., 2005).

Because of this reason, the present study explored the variability of genetic resistance to *Fusarium* ssp. and investigated the accumulated toxins in a set of modern cultivars used as inoculated and non-inoculated standard cultivars.

MATERIAL AND METHODS

The experimental biologic material consisted in eleven modern standard cultivars (Argentina, Auteuil, Belinda, Evora, Genziana, Ivory, Jaak, Krezus, Mures, Mina and Saul as naked cultivars). The used standard cultivars (Table 1) represent a wide range of European

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breeding traditions. They were acquired and provided by partners in Sweden, Estonia, France, Germany, Italy, Poland, Romania and Bulgaria.

Table 1. Standard cultivars used in five replications

Represented Region	Cultivar	Breeder	Represented Region	Cultivar	Breeder
Northern Europe	Belinda	Svalöf Weibull	Southern Europe	Argentina	SIS
	Jaak	Jõgeva PBI		Genziana	CRA-ISCI
Western Europe	Auteuil	Serasem	Eastern Europe	Krezus	Strzelce
	Evora	Serasem		Saul	Selgen
Central	Ivory	Nordsaat	South Eastern Europe	Mures	Turda
				Mina	Malkow

Investigations were conducted in the experimental field of the Suceava Genebank (2008) in five inoculated replications and two non-inoculated replications.

The inoculation was accomplished with inoculum produced by Julius Kuehn Institute (Germany) using isolates provided by the JKI collection in Berlin Dahlem from five species *Fusarium culmorum, Fusarium graminearum, Fusarium langsethiae, Fusarium sporotichioides, Fusarium avenaceum* multiplied on different substrates (Table 2).

Table 2. Fusarium species and multiplied isolates used for inoculation

Species	Substrate	Country	Number of spores / 2070 g
Fusarium graminearum, Scwabe	*Triticum aestivum*	Germany	24812462
Fusarium culmorum (W.G. Smith) Saccardo	*Secale cerealis*	Germany	8686408502
Fusarium avenaceum (Corda:Fries) Saccardo	*Hordeum vulgare*	Germany	120111728
Fusarium sporotrichioides Var. minus Wollenweber	*Avena sativa*	Germany	2578796550
Fusarium langsethiae Torp Nirenberg	*Avena sativa*	Austria	6521739000

The obtained suspension which resulted after washing the infested grains was sprayed early morning on panicles, during June 16th - July 14th 2008 interval, three times, when of the plants flowering phenophase.

In order to ensure an optimum moisture level, the plots were irrigated two days before and after inoculation.

The panicles infection with *Fusarium* head blight was visually assessed using the 1-9 scale (1- no infection, 9- all spikelets and panicles infected).

The percentages of infected seeds with *Fusarium* sp. were evaluated using magnifying glass. Three evaluation classes were used: 1. sound, 2. suspiciously infected, grey tips, 3. *Fusarium* damaged, discolored, smaller kernels.

The accumulated mycotoxins on inoculated and non-inoculated standard (1 repetition) were analyzed in Eurofins laboratory Contaminants WEJ GmbH, Hamburg, Germany by the method of LC-MS/MS (Biselli and Hummert, 2005). For analyzing each standard genotype, about 20 g of flour from non dehuling grains were used in accordance with European rules (Regulation (EC) No 1881/2006).

Based on the EU limits for contaminants in foodstuffs (Commission Regulation EC 1881/2006) *Deoxynivalenol* (DON), *Zearaleone* (ZEA) and *Trichothecenes* (T-2 and HT-2 toxin) should be analyzed. Limits for these toxins in cereal products are shown in Table 3.

Table 3. Limits for *Fusarium* toxins in cereal products (by Regulation (EC) 1881/2006)

Specification	DON [ppb]	ZEA [ppb]	T2+ HT2 [ppb]
Unprocessed cereals	1250	100	100
Unprocessed oats	1750	100	500
Cereals intended for direct human consumption like cereal flour, *oat products	750	75	200*

RESULTS AND DISCUSSION

The trichotecenes B (deoxynivalenol, nivalenol) and zearalenone caused by *Fusarium graminearum* and *Fusarium culmorum*, the trichotecenes A (T2, HT2) caused by *Fusarium sporotrichioides* and *Fusarium langhsethiae*, were analyzed in a single repetition by LC-MS/MS method in inoculated and non-inoculated genotypes.

The distribution of mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) was uneven for both variants (inoculated and non-inoculated), but arithmetic mean and median values indicate higher concentrations in non-inoculated variants (Table 4). Concerning vomitoxin and nivalenol (NIV), T2, HT2 mycotoxins, they were evenly distributed in both variants, the arithmetic mean and median values highlighting increased concentrations only in inoculated variants (Table 4).

Table 4. Mycotoxin contents for standard cultivars grown both under conditions of natural and artificial infection in 2008, analyzed in the Eurofins laboratory WEJ Contaminants GmbH, Hamburg, Germania by LC-MS/MS method

Accession number	Cultivar name	Inoculated standards mycotoxin (μg kg^{-1})					Non-inoculated standards mycotoxin (μg kg^{-1})				
		DON	T2	HT2	ZEA	NIV	DON	T2	HT2	ZEA	NIV
00004	Jaak	31	138	288	0	450	45	43	118	0	440
8	Argentina	160	158	819	72	1400	72	154	465	0	230
700	Genziana	100	66	304	0	2400	120	156	362	0	270
30040	Auteuil	45	13	266	0	750	49	39	159	0	120
A7BM0001	Mina	65	8	45	0	74	0	7	34	0	110
CPVO19960125	Belinda	50	36	188	0	430	58	16	136	0	33
CPVO19981528	Evora	0	39	131	0	490	60	70	274	0	310
CPVO20040091	Ivory	0	125	465	12	760	220	82	276	14	330
Krezus	Krezus	0	81	440	0	870	46	33	147	0	240
ROM007-16701	Mures	143	19	144	0	290	20	19	129	0	290
Saul	Saul	0	10	400	0	35	220	0	400	95	0
	Mean	54	63	317	7.6	722	82.7	56.2	227.2	9.9	216
	Median	45	39	288	0	490	58	39	159	0	240
	Min.	0	8	45	0	35	0	0	34	0	0
	Max.	160	158	819	72	2400	220	156	465	95	440

Mycotoxins T2 and HT2 are showing a higher concentration in inoculated variants (max. 158 μg kg^{-1} T2, 819 μg kg^{-1} HT2) compared with noninoculated variants (max. 156 μg kg^{-1} T2, 465 μg kg^{-1} HT2), below the accepted values by European rules (Figure 1).

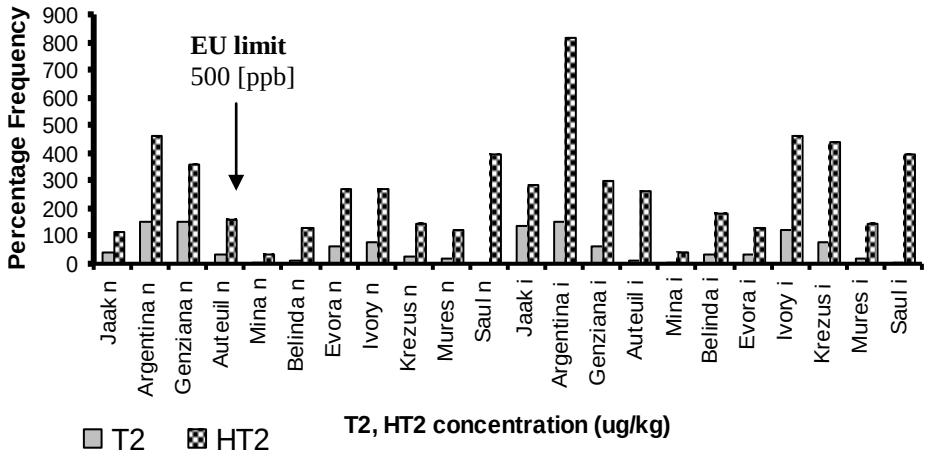

Figure 1. Percentage frequency of HT2, T2 contamination in inoculated and noninoculated standard

The trichothecene B (deoxynivalenol) and zearalenone showed a higher level of concentration in noninoculated variants (max. 220 mg kg^{-1} DON, 95 µg kg^{-1} HT2) than those inoculated (max. 160 µg kg^{-1} DON, 72 µg kg^{-1} HT2), both under the limit allowed by the European Commission (1881/2006), (Figure 2).

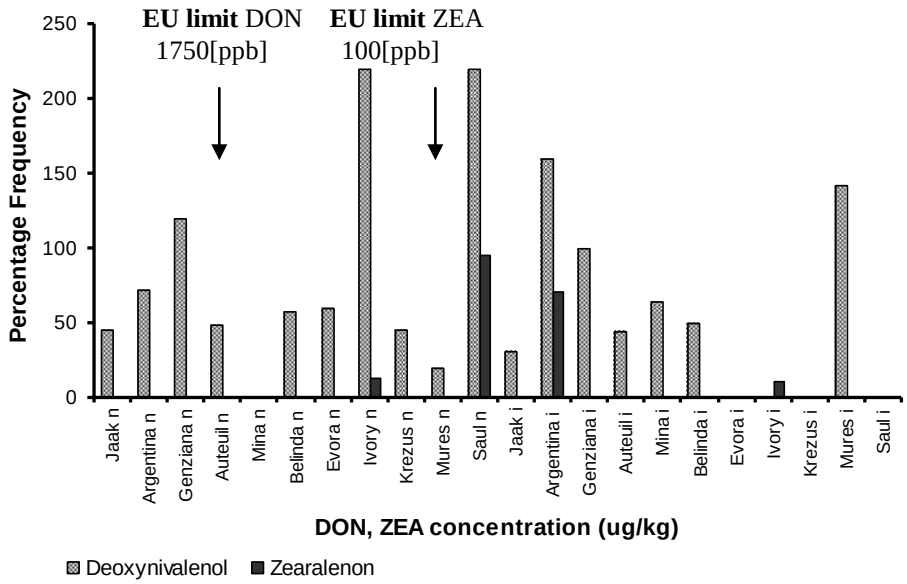

Figure 2. Percentage frequency of DON, ZEA contamination in inoculated and noninoculated standard

The statistical correlation (Table 5) between the incidence of *Fusarium* micromycetes on panicles (FHB), on harvested grains (FDK class 3) and mycotoxins contents from the inoculated and non-inoculated standard variants showed statistically very significant values between DON and ZEA (0.644**), ZEA and HT2 (0.573**) and between T2 and HT2 0.682**) and low significant values between FDK (*Fusarium* damaged kernels) class 3 and zearalenone (0.526*).

Table 5. Coefficient of correlations (SPSS) between *Fusarium* incidence and mycotoxins concentration in inoculated and non-inoculated standards (Pearson - coefficient of correlation; bold = significant)

	FHB	FDK score 3	Deoxynivalenol	Zearalenon	T2	HT2	Nivalenol
FHB	1.000	0.165	-0.299	0.058	-0.136	-0.035	0.202
P	-	0.462	0.176	0.797	0.545	0.876	0.367
FDK score 3	0.165	1.000	0.169	**0.526***	-0.395	0.152	-0.267
P	0.462	-	0.453	0.012	0.069	0.500	0.230
Deoxynivalenol	-0.299	0.169	1.000	**0.644****	0.144	0.309	0.077
P	0.176	0.453	-	0.001	0.523	0.162	0.734
Zearalenone	0.058	**0.526***	**0.644****	1.000	0.090	**0.573****	0.085
P	0.797	0.012	0.001	-	0.690	0.005	0.707
T2	-0.136	-0.395	0.144	0.090	1.000	**0.682****	0.338
P	0.545	0.069	0.523	0.690	-	0.000	0.124
HT2	-0.035	0.152	0.309	**0.573****	**0.682****	1.000	0.421
P	0.876	0.500	0.162	0.005	0.000	-	0.051
Nivalenol	0.202	-0.267	0.077	0.085	0.338	0.421	1.000
P	0.367	0.230	0.734	0.707	0.124	0.051	-

Figure 3 shows the regression line between contamination level by *Fusarium* sp. (FDK class 3) and zearalenone accumulation in grains.

In Figures 4, 5 and 6, the positive correlations between concentrations of mycotoxins ZEA and HT2, ZEA and DON, T2 and HT2, existing in inoculated and noninoculated standards, are represented by regression lines, showing an increase directly proportional to the accumulation of these groups of mycotoxins in grains, the aggresiveness of competing resulted *Fusarium* species having an important role.

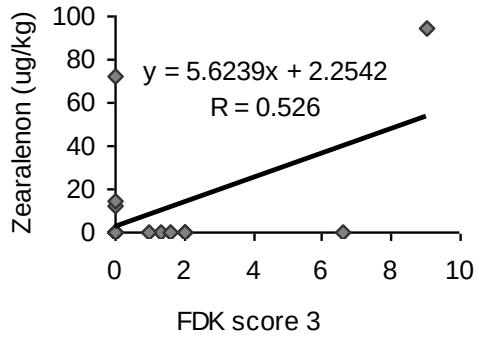

Figure 3. FDK score 3 against ZEA from inoculated and noninoculated cultivars

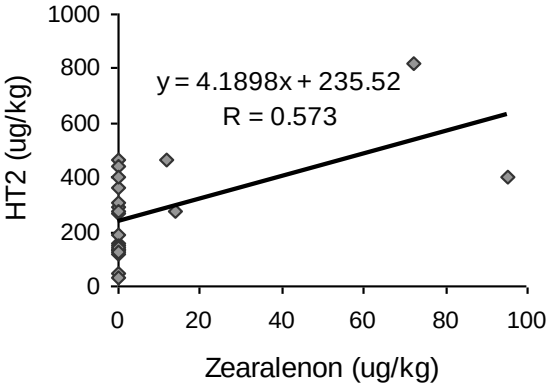

Figure 4. ZEA against HT2 from inoculated and noninoculated cultivars

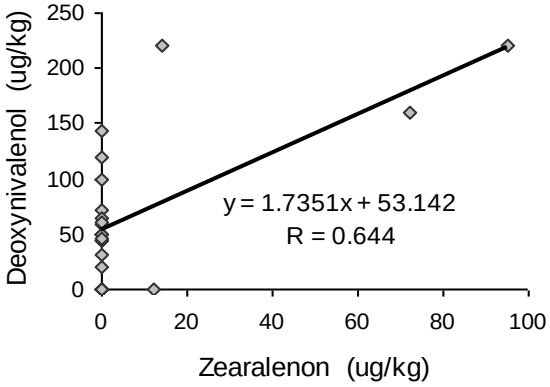

Figure 5. ZEA against DON from inoculated and noninoculated cultivars

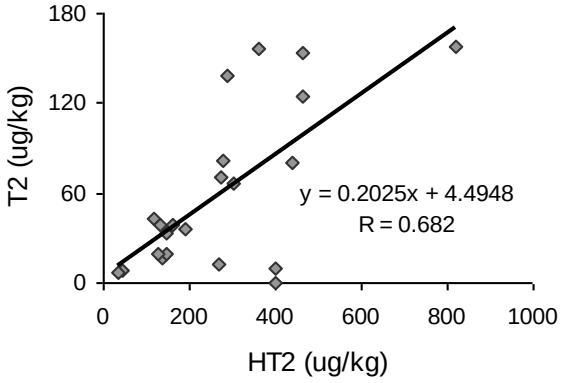

Figure 6. HT2 against T2 from inoculated and noninoculated cultivars

CONCLUSIONS

The analyses accomplished by LC-MS/MS method for determination of the deoxynivalenol (DON), zearalenon (ZEA) and nivalenol, T2, HT2 mycotoxins, outlined their presence below accepted European rules limits (1881/2006) in both naturally infected and inoculated standard variants.

Inoculation with a mixture of *Fusarium* sp. fungi has significantly influenced the content of trichotecine B (nivalenol), trichotecine A (T2 and HT2) and substantially the content of deoxynivalenol and zearalenone in modern standard cultivars.

The natural infection in standard variants determined high concentrations of trichotecine HT2 (average 227.2 μg kg^{-1}) in comparison with other mycotoxins like nivalenol (average 216 μg kg^{-1}), deoxynivalenol (average 82.7 μg kg^{-1}) and zearalenone (average 9 μg kg^{-1}) .

The incidence of *Fusarium graminearum* and *Fusarium culmorum* determined the highest content of nivalenol (average 722 μg kg^{-1}) in inoculated genotypes and deoxynivalenol, zearalenone in naturally infested genotypes.

Correlation between infection with *Fusarium sp.* (FDK-score 3) on grains and the contamination with mycotoxins revealed a low influence of infection degree with zearalenone in grains.

Statistically significant relationships between concentrations of mycotoxins ZEA and HT2 (0.573*), ZEA and DON (0.644**), T2 and HT2 (0.682**) from the inoculated and non-inoculated standards showed that these groups of mycotoxins interact directly proportional with concentration increases on the grains. An important role in this phenomenon had the species of *Fusarium* that produced mycotoxins in different environmental conditions (natural or artificial), as well as the competition between the aggressiveness of each species in the same ecological niche.

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