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FUSARIUM SPP. AND MYCOTOXIN ANALYSIS ON OAT GENETIC RESOURCES

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ABSTRACT

The analysis of oat genetic resources on the mycotoxin production was performed on genotypes grown by experimental field testing with artificial inoculation of *Fusarium* spp., within the European project "Avena genetic resources for quality in human consumption" (AVEQ, 2007-2011).

The testing of the genetic material was performed in three European countries (Romania, Czech Republic, Germany) by artificial infection with inoculum of *Fusarium* species*: F. culmorum, F. graminearum, F. sporotichioides, F. langesethiae* and *F. avenaceum*.

In 2008, in the experimental field of the Suceava Gene Bank, 112 genotypes of oats (modern and obsolete cultivars) originating from different areas of Europe were inoculated, causing *Fusarium* spp. infection and the presence of DON and T2 mycotoxins.

The infection of oat grains with *Fusarium* spp. was evaluated in the laboratory using three classes of infection: 1- sound, 2- suspiciously infected, grey tips, 3 - *Fusarium* damaged, discoloured, smaller kernels.

Analysis of the mycotoxin content of the tested grains oat accessions was performed by the ELISA immunochemical method in the Agrotest Fyto Laboratory, Ltd., Kromeriz (Czech Republic).

The quantification of the obtained results was made by determining the correlation coefficients between the mycotoxin content and the percentage of grains infected with the *Fusarium* spp. and different agronomic characters, revealing significant correlations between *Fusarium* spp. infection, concentration of DON and T2 mycotoxins, the seed weight, heading and ripening data of tested oat genotypes.

Key words: *Fusarium* damaged kernels (FDK), mycotoxins, genotypes, inoculation, evaluation

INTRODUCTION

Oat has been more often used in human nourishment in recent years because of the high amount of dissoluble fibre, especially β-glucan in its kernels that is considered to be necessary for any rational diet (Wood et al., 1990).

Fusarium spp. are pathogens directly affecting oat panicles and kernels (Veisz et al., 1997). Infection of plant panicles by fungi of *Fusarium* genus has a direct negative effect on both the yield size, causing its decrease, and the quality of the kernels (Kiecana, 1994; Langseth et al., 1995; Mielniczuk, 2001; Kiecana et al., 2002). Besides, as a result of infection by *Fusarium* spp., accumulation of mycotoxins, which are harmful to human and animals, takes place in the infected kernels (Vesonder & Golinski, 1989; Perkowski et al., 1997; Goli´nski et al., 1999).

According to Tekauz et al. (2004) *Fusarium* spp. usually is not apparent in a field of oats, and unlike the situation in wheat (in particular) and barley, visual in-crop severity is not a valid indicator of the damage to mature seed. Despite panicle symptoms are not obvious and seed and test weights of the harvested grain are good, high levels of T2 and HT2 can be observed.

The amount of mycotoxins will vary depending on timing of initial infection, environmental conditions, cereal species and cultivar resistance (Agriculture and Agri-Food Canada, 2004). Exploring the available literature there is not much information about the real toxin accumulation resistance in oats and the genetic variability for resistance to *Fusarium* in cultivated and wild oats.

Because of limited information, the present study investigate 112 oat genotypes (modern and obsolete cultivars) after panicles inoculation with isolates of five species of *Fusarium*, emphasizing the effect of fungus in contaminated kernels and the mycotoxins accumulation in relation to some agronomical descriptors.

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MATERIAL AND METHODS

The experimental biologic material consisted in 112 oat genotypes (31 modern cultivars, 11 standard modern cultivars and 70 obsolete cultivars). The used cultivars (Table 1) represent a wide range of European breeding traditions. They were acquired and provided by partners in Sweden, Lithuania, Slovakia, Russia, Estonia, France, Germany, Italy, Poland, Czech, Romania and Bulgaria.

Table 1. Modern and obsoletes cultivars acquired from breeders for field experiments (Suceava, 2008)

Represented region	Modern cultivar	Breeder/ registration	Obsoletes cultivar	Breeder/ registration
Northern Europe	7	Svalöf (5)/ 1996-2007 Jogeva (2)/ 1991-1999	12	Unknown breeder/ Registration before 1900, 1900- 1960
Western Europe	18	Edelhof (3), Saatbau (2)/ 1992-2006 Serasem (5)/ 1992-2007 Nordsaat (5), Lochow-Petkus (3)/ 2000-2008	22	
Eastern Europe	15	Danko (2), Malopolska (2), Strzelce (3)/ 1992-2008 Moldovan (1)/ 1991 Obraztcov Chiflik 4 (1), Selgen (6)/ 1988-2008	34	
Southern Europe	2	CRA- ISCI (2)/ 1999-2005	2	

Investigations were conducted in the experimental field of the Suceava Genebank, on 320 accessions and 11 standards in five replications. The genotypes were sowed during 8-10 April 2008, in the twelve blocks with forty plots each. Sowing rate was 400 seeds m^{-2} on the plots with surface of 2.5 m^2. During the vegetation period herbicide (Ceredin Super 40 SL) and fertilization treatment (ammonium nitrate) were used.

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 resulted after washing the infested grains was sprayed early morning on panicles, during June 16th - July 14th 2008 interval, three times, when most of the plants were in flowering phenophase. In order to ensure an optimum moisture level, the plots were irrigated two days before and after inoculation. From the 320 artificially inoculated accessions, only 112 low-*Fusarium* genotypes were proposed in this study for mycotoxins determination.

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

The mycotoxins concentration on inoculated cultivars were analyzed in Agrotest Fyto laboratory, Ltd., Kroměříž (Czech Republic) by immunochemical method, that is based on the ELISA principle (Enzyme Linked Immune Sorbent Assay). Methods based on immunoassay principles are now among the most widely used methods to screen for and quantify some of *Fusarium* mycotoxins in the grain industry (Maragos and Platner, 2002). Yang et al. (2003) reported that ELISA is suitable to analyze even baby food and cereal samples in accordance with the tightening European legislative limits for DON.

The limit of detection of the mycotoxin concentration level that can be measured with ELISA method is for DON < 20 ppb and T-2 <5 ppb. For analyzing each cultivar, about 20 g of flour from non dehulled grains were used in accordance with European rules (Regulation (EC) No 1881/2006).

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

Specification	DON [ppb]	T-2+ HT-2 [ppb]
Unprocessed cereals	1250	100
Unprocessed oats	1750	500
Cereals intended for direct human consumption like cereal flour, *oat products	750	200*

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

RESULTS AND DISCUSSION

The obtained results from the immunological testing of artificially infected grains of *Fusarium* spp. revealed high values of DON concentrations in obsolete (1098 µg kg^{-1}) and standards (528 µg kg^{-1}) cultivars compared to modern cultivars.

Also, tricotecine T2 had higher maximum values in standards (260 µg kg^{-1}) versus modern (158 µg kg^{-1}) and obsolete (237 µg kg^{-1}) genotypes. In poorly susceptible cultivars with 1-1.5% infection degree of FDKs, both mycotoxins showed concentrations of minimum and maximum on 0-1098 µg kg^{-1} for DON, and 8 to 260 µg kg^{-1} for T2, respectively.

Mean concentrations of mycotoxins have a higher DON and T2 conductivity level in obsolete (DON-188 µg kg^{-1}, T2- µg kg^{-1}) and standards (DON-165 µg kg^{-1}, T2-95 µg kg^{-1}) cultivars, compared to modern ones (Figure 1), but below the limits allowed by the European Commission (Table 4).

Table 4. The % *Fusarium* damaged kernels and mycotoxin contents for 112 cultivars grown under artificial infection conditions in 2008, analysed in the Agrotest Fyto laboratory, Ltd., Kroměříž (Czech Republic) by ELISA method

Cultivars	Cultivars number	ELISA-DON (µg kg^{-1})			ELISA-T2 (µg kg^{-1})			*Fusarium* damages kernels (FDK) %		
		Min	Average	Max	Min	Average	Max	Min	Average	Max
Standard	11	22	**165**	528	32	**95**	260	0,3	**1.5**	4.7
Modern	31	0	**54**	160	8	**63**	158	0	**1.2**	2.3
Obsolete	70	10	**188**	1098	24	**87**	237	**0**	**1**	2.3

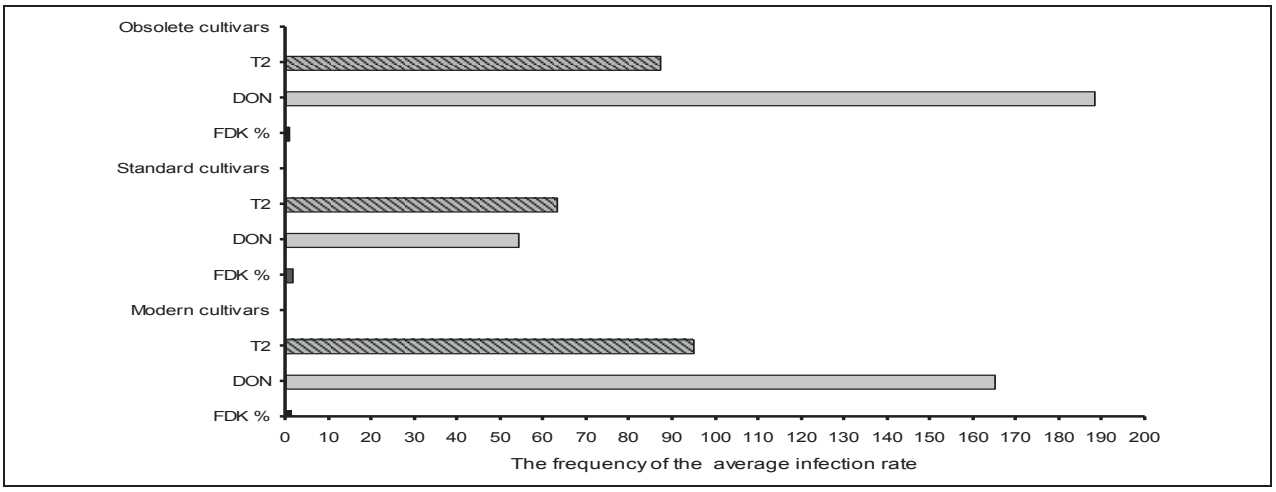

Figure 1. The mycotoxin concentration of DON, T2 and percentage of *Fusarium* damaged kernels (average value from inoculated cultivars)

With SPSS statistical program, the Pearson correlation coefficients between *Fusarium* grain infection (FDK%), the concentration of DON and T2 mycotoxins, and some agronomic descriptors (test weight, seed weight, days to heading, days to maturity, panicle length) were determined. In Table 5 negative significant correlation coefficients between DON and FDK%; significant positive between T2 and days number to maturity; distinctly significant between DON and T2; T2 and days number to heading; insignificant between the T2 and panicle length; T2 and test weight respectively are presented.

Table 5. Correlations coefficient of (SPSS) between *Fusarium* incidence, agronomical traits and mycotoxins concentration on the 112 inoculated cultivars (P- Pearson coefficient of correlation; bold sig. (2-tailed)* - is significant at the 0.05 level, ** - is distinctly significant at the 0.01 level)

Specification	FDK %	DON (μg kg^{-1})	T2 (μg kg^{-1})	Test weight	Seed weight	Days to heading	Length to panicle	Days to maturity
FDK % P	1	**-0.193***	0.071	-0.031	0.037	0.020	0.011	0.120
Sig.(2-tailed)		0.041	0.455	0.746	0.698	0.838	0.911	0.209
No.cultivars	112	112	112	112	112	112	112	112
DON P	**-0.193***	1	**0.307****	0.047	-0.071	0.050	0.053	0.044
Sig. (2-tailed)	0,041		0.01	0.620	0,459	0.602	0.577	0.644
No. cultivars	112	112	112	112	112	112	112	112
T2 P	0,071	**0.307****	1	0.080	-0.210	**0.264****	0.074	**0.237***
Sig. (2-tailed)	0.455	0.01		0.400	0.026	0.005	0.441	0.012
No.cultivars	112	112	112	112	112	112	112	112
Test weight P	-0.31	0.047	0.080	1	0.038	-0.121	0.098	-0.115
Sig. (2-tailed)	0.746	0.620	0.400		0.691	0.204	0.303	0.226
No.cultivars	112	112	112	112	112	112	112	112
Seed weight P	0.037	-0.071	**-0.210***	0.038	1	**-0.273****	-0.118	-0.234*
Sig. (2-tailed)	0.698	0.459	0.026	0.691		0.004	0.217	0,013
No.cultivars	112	112	112	112	112	112	112	112
Days to	0.020	0.050	**0.264****	-0.121	**-0.273****	1	0.117	**0.858****
heading P	0.838	0.602	0.005	0.204	0.004		0.221	0.000
Sig. (2-tailed) No.cultivars	112	112	112	112	112	112	112	112
Lenght to	0.011	0.053	0.074	0.098	-0.118	0.117	1	0.110
panicle P	0.911	0.577	0.441	0.303	0.217	0.221		0.250
Sig. (2-tailed) No.cultivars	112	112	112	112	112	112	112	112
Days to	0.120	0.044	**0.237***	-0.115	**-0.234***	**0.858****	0.110	1
maturity P	0.209	0.644	0.012	0.226	0.013	0.000	0.250	
Sig. (2-tailed) No.cultivars	112	112	112	112	112	112	112	112

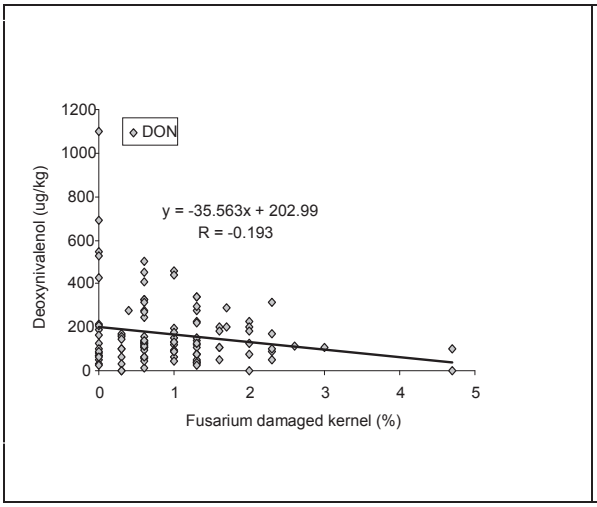

Figure 2. The regression line for the correlation between FDK % (*Fusarium* damaged kernels) and DON µg kg^{-1} (Deoxynivalenol)

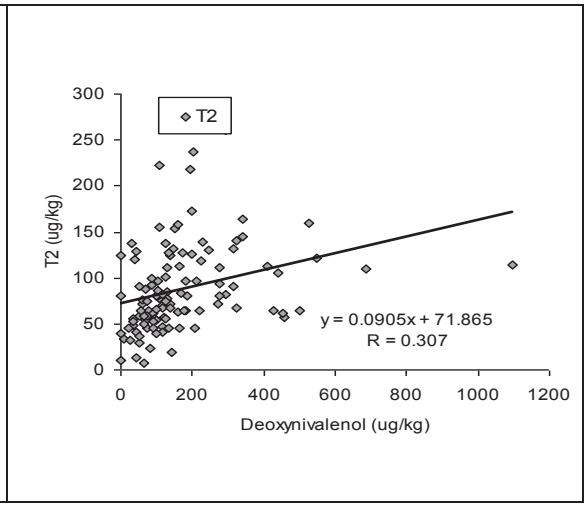

Figure 3. The regression line for the correlation between T2 µg/kg toxin and DON µg kg^{-1} (Deoxynivalenol)

The negative relation (-0.210) between T2 and seed weight is also evidenced by the descendent regression line (Figure 4); increased concentrations of T2 being found in cultivars with small seed weight.

The correlation coefficient between DON concentration and *Fusarium*-infected grains (FDK%) is negative significant (-0.193*), being represented by a descendent regression line (Figure 2), the concentration of DON mycotoxins having high values in infected cultivars.

Between the presence of T2 mycotoxin and the number of days to maturity there is a positive significant correlation (0.237*); the ascending regression line (Figure 4) showing an increase of the T2 mycotoxin concentratation in the late cultivars. The distinctly significant correlations between DON and T2 mycotoxins (0.307**), and the number of days to heading (0.264**), show a strong relation between mycotoxins produced by different species of *Fusarium* spp. and an increase of tricotecine T2 concentration during heading period.

In Figure 3 the regression line highlights that the content of T2 mycotoxin is correlated with the increase of DON toxin concentration. The values of tricotecine T2 increased in cultivars with a larger number of days to heading, as showed by the ascending tendency of the regression line (Figure 4).

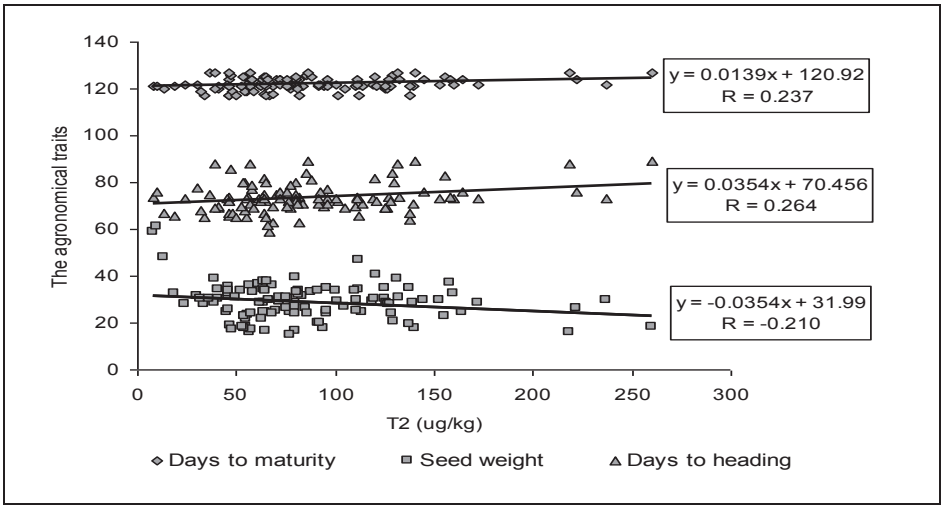

Figure 4. The regression lines for the correlation between T2 and some agronomical traits (days to heading, days to maturity, seed weight)

CONCLUSIONS

The oat cultivars inoculated with *Fusarium* spp. and tested by the ELISA immunological method, revealed the presence of tricotecins B-DON (Deoxynivalenol) and Tricotecins A-T2 below the limit allowed by the European Commission (Regulation (EC) No 1881/2006).

In obsolete cultivars the maximum value of DON was 1098 µg kg^{-1} and of T2 was 237 µg kg^{-1}. In standard cultivars the maximum value was 528 µg kg^{-1} for DON and 260 µg kg^{-1} for T2 mycotoxin, the genotypes studied being low susceptible to *Fusarium* infection (1-1, 5 %).

A significant correlation (-0.193*) between the susceptibility of cultivars to *Fusarium* and the DON mycotoxin concentration, was revealed.

A strong relationship was found between mycotoxins produced by different species of *Fusarium* spp., with a distinct significant correlation between deoxynivalenol (DON) and T2 (0.307**), the content of mycotoxins T2 being correlated with the increase of DON toxin.

The significant correlations between the T2 concentration and some agronomic traits such as: seed weight (-0.210*), days to heading (0.264**), days to maturity (0.237*) representing a significant increase of tricotecin A (T2) in cultivars with small values of seed weight, and in tardive cultivars.

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