PRELIMINARY EVALUATION OF SOME FACTORS INVOLVED IN DON CONTAMINATION OF BREAD WHEAT UNDER NATURAL AND ARTIFICIAL INOCULATION

Mariana Ittu[1], Lidia Cană[1], Cristina Tabuc[2], Ionelia Țăranu[2]

ABSTRACT

Natural occurrence of DON accumulation in grains of bread winter wheat sampled from eight locations x two years, and relations between DON content and some phenotypic FHB traits, under field artificial inoculation with *Fusarium graminearum* and *F. culmorum* isolates were evaluated. Quantification of DON content under natural conditions revealed differences between locations, years and genotype/environment interactions. DON contents reaching or overcoming accepted limits for food processing (1.25 ppm) were found in some environments. Efficiency of a higher level of FHB resistance *vs.* DON contamination under artificial inoculation was demonstrated. In some environments, phenotypic traits, mainly percentage of FDK could be a good indicator associated with DON contamination of grains. Further breeding efforts are required to build genotypes combining both traits, a low content of DON and low FDK, %.

Key words: *Fusarium* head blight (FHB), Deoxynivalenol (DON), grain contamination, severity (SEV), area under disease progress curve (AUDPC*),* *Fusarium* diseased kernels (FDK).

INTRODUCTION

Fusarium head blight (FHB or scab) caused by several *Fusarium* species among which *Gibberella zeae* (Schwein.) Petch. (anamorphs, *Fusarium graminearum* and *F. culmorum*) is the most reported, continues to be a disease of world-wide concern in wheat.

The unpredictable damaging effects of this disease consist in severe yield and grain quality losses, very often associated with mycotoxins accumulation, rendering it unsuitable for human and livestock consumption (Gilbert and Tekauz, 2000; Leonard and Bushnell, 2003). Trichotecenes are the most prominent group of mycotoxins produced by *Fusarium*, deoxynivalenol (DON) being the most prevalent one (Placinta et al., 1999).

DON is considered a virulence factor that facilitates colonization of heads during FHB development (Bai et al., 2001), and inducing similar disease symptoms in wheat (Ittu et al., 1995; Lemmens et al., 2005). Cellular effect of DON includes inhibition of protein synthesis, alteration of plasma membrane permeability, chloroplast dissolution and induction of plant programmed cell death (PCD) (Bushnell et al., 2004). Increased losses caused by *Fusarium* diseases were registered in the past decades after wider adoption of minimum tillage practices and wheat/maize rotation, as far as both crops are alternative hosts for this fungus (Dill-Macky and Jones, 2000). Climate change is also likely to increase FHB occurrence in some parts of the world. Development of resistant cultivars has been a primary thrust of research efforts made by public and private breeding programs all over the world, in order to reduce the economic impact of FHB on wheat industries. Initially, most programs focused on reducing FHB in the crop targeted by transfer of resistance Type 1 and Type 2 into adapted wheat backgrounds. This process was accelerated in recent years by discovery of diagnostic markers for the most reported QTL that made MAS available (Buerstmayr et al., 2008). Growing concern of consumers for food and feed safety translated attention given by policy makers to food safety. New regulations recently enforced by the EU (Regulation EC no. 1126/2007 of 28 September 2007) limit DON level in wheat grains (1.25 ppm = 1.25 mg/kg), flour (0.75 ppm) and end products (0.2-0.5 ppm). As a consequence the emphasis has shifted to reducing DON and *Fusarium* damaged kernels (FDK) (Duveiller et al., 2008). The inherent problems associated with phenotyping of FHB resistance and DON contamination of grains consist of the difficulty in replicating selection environments. Poor or late development of FHB may result in underestimates of severity and/or low correlation with FDK and DON concentration (Brown-Guedira et al., 2008). In Romania, damaging attacks induced by *Fusarium* pathogens in wheat and maize, the most important staple food crops, cultivated on large acreages, were constantly registered (Ittu, 2001; Ittu et al.,

[1] National Agricultural Research and Development Institute Fundulea, 1 N. Titulescu street, 915200 Fundulea, Călăraşi County, Romania
[2] National Institute for Biology and Animal Nutrition Baloteşti , 1 Calea Bucureşti, 077015 Baloteşti, Romania

2004). However, only a few reports on natural occurrence of DON contamination are available, but evidence of higher concentrations of DON in wheat and maize samples from western region was reported (Curtui et al., 1998).

The objectives of this study were to evaluate factors involved in DON accumulation of wheat grains, under natural and artificial inoculation, with the aim to improve knowledge on occurrence of DON in wheat cropping conditions of Romania.

MATERIAL AND METHODS

Plant materials. 80 grain samples of ten winter bread wheat varieties, randomly harvested from yield trials grown under natural conditions, without artificial inoculation, in eight locations representative for wheat crop across Romania were analyzed for two years (2006-2007). In a second experiment conducted in the same period, samples of three genotypes with different levels of resistance to FHB, ranging from medium resistant (Fundulea 201R) to very susceptible (Fundulea 133), were phenotyped and analyzed for DON content, following artificial field inoculation with either of four *Fusarium graminearum* and *F. culmorum* isolates, in FHB nurseries at NARDI-Fundulea.

Methods for artificial inoculation experiment included:

***Fusarium* isolates.** A single-spore isolates of *F. graminearum* (FG 96, FG 54, FG 111) and *F. culmorum* (FC 46), originally isolated in Romania and in The Netherlands, respectively were separately used for inoculation. FC 46 was kindly provided by T. Miedaner (Miedaner et al., 2001). Inoculum was a suspension of conidia produced on wheat grains (14 days at 25 °C in dark), dried for up to seven days under exposure to black UV lamps (Philips HPL-N 400W E40) at room temperature.

Artificial inoculation. Wheat genotypes were artificially field point inoculated at anthesis. Approximately 10 µl droplet was injected by a self-refilling syringe Type Dosys™ classic 173, directly through the glumes in a central floret of each side of 20 arbitrarily chosen heads per entry that were distinctly marked to allow repeated ratings.

FHB phenotypic traits. Scoring of FHB started in field 10 days post inoculation (pdi) and repeated at 20 dpi in terms of infected spikelets/entry/isolate. The arithmetic mean of the individual successive ratings was used for further calculation of severity, SEV (damaged spikelets, % of control at the onset of symptom development, i.e. 20 dpi), and disease progress, area under disease progress curve (AUDPC). At full ripening, inoculated spikes/entry/isolate were harvested and threshed by hand, to save highly infected, shriveled and degenerated kernels. From these samples percentage of *Fusarium* diseased kernels (FDK, %) was appreciated.

DON immunoassay analysis. Grains from heads both randomly harvested under natural conditions and artificially inoculated, were bulked, ground and analyzed for DON content at the National Institute for Biology and Animal Nutrition Baloteşti, Romania. The concentration of DON was quantified according to the manufacturer's description by VERATOX DON HS (NEOGEN Quantitative High Sensitivity Test, U.S., LOQ = 25 ppb) and expressed in ppm (parts per million).

Statistical analysis. Correlation analysis was performed for phenotypic FHB traits and DON content of grains.

RESULTS AND DISCUSSION

Factors of DON accumulation under natural condition. Quantification of DON content in wheat samples, revealed values not exceeding on average, the admitted level of contamination for unprocessed production, respectively 1.25 ppm (1250 µg/kg), for the period of two years investigated. Higher maximum concentrations of DON were found in samples from Livada (3.3 ppm), Braşov (1.72 ppm) and Turda (1.3 ppm), generally characterized by endemic occurrence of FHB (Figure 1).

DON analysis of ten varieties sampled across two combinations year/location (two locations, two years) showed frequencies of detectable DON concentrations that ranged from 70 to 85% on average/year.

DON accumulation varied from 0-1.72 ppm (0.69 ppm, on average) at Braşov, while this parameter was quite low at Fundulea, 0-0.37 ppm (0.16 ppm, on average), respectively. On the contrary, in both locations insignificant values of DON accumulation were found in 2007 (0.05 ppm on average), even in the case of varieties previously characterized as higher contaminated (Table 1). In favorable conditions for FHB development, levels of grain contamination with the associated mycotoxin DON could reach or overcome accepted limits for food processing (1.25 ppm). In order to properly classify currently cultivated varieties with respect to their resistance to DON, further confirmations from independent trials are necessary. These findings suggest the importance of environment (year and location) and cultivar/environment interaction on DON contamination under natural conditions and the necessity to permanently monitor the FHB attack, mainly in the regions under frequent risks of infection with *Fusarium* pathogens and *Fusarium* toxigenic strains.

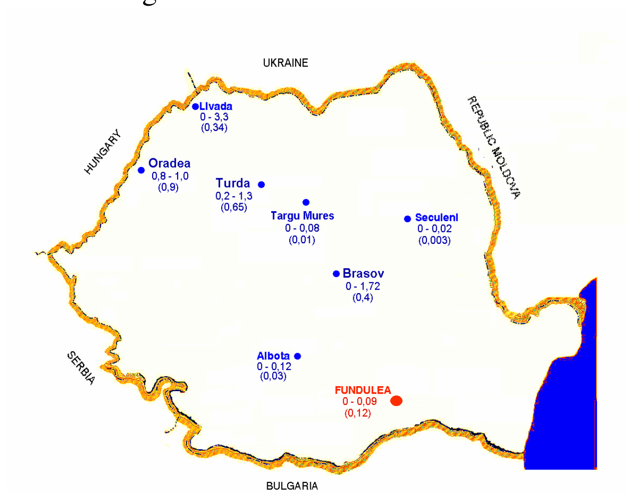

Figure 1. DON, ppm content of wheat grains in eight locations of Romania: range of variation and mean values across ten varieties/location

Table 1. Frequency, minimum, maximum, mean values of DON content in ten wheat varieties (two locations and two years)

Variety	DON, ppm						
	2006			2007			Average
	Braşov	Fundulea	*Average*	Braşov	Fundulea	*Average*	2006/2007
Ariesan	1.17	0.21	*0.40*	0.03	0.21	*0.12*	0.26
Bezostaia	0.26	0.07	*0.17*	0	0.01	*0.01*	0.09
Boema	0.62	0	*0.31*	0	0	*0*	0.16
Crina	0	0	*0*	0.05	0.03	*0.04*	0.02
Dor	1.72	0	*0.86*	0.01	0.06	*0.04*	0.45
Delabrad	1.02	0.21	*0.62*	0.08	0.04	*0.06*	0.34
Dropia	0	0	*0*	0.01	0.05	*0.03*	0.02
Dumbrava	0.78	0.08	*0.43*	0.02	0.02	*0.02*	0.23
Gruia	0.62	0.7	*0.66*	0.02	0.20	*0.11*	0.39
FL 85	0.72	0.37	*0.55*	0.04	0.03	*0.04*	0.29
Frequency, %	80	60	*70*	80	90	85	77.5
Average	*0.69*	*0.16*	*0.43*	*0.03*	*0.07*	*0.05*	0.24
Minimum	0	0	*0*	0	0	0	0
Maximum	1.72	0.37	*0.86*	0.08	0.21	*0.12*	0.45

Factors of DON accumulation under artificial inoculation. Ratings of some FHB phenotypic traits as severity, area under disease progress curve (AUDPC) and percentage of *Fusarium* diseased kernels and DON content, showed differences among both wheat cultivars and cultivar/environment interactions.

Although FHB symptoms were successfully reproduced in both environments, significant higher DON content was quantified on average in 2006 (241 ppm), while the corresponding data for 2007, indicate a reduced level of DON accumulation, 0.9 ppm on average, respectively (Table 2). Even in more favorable environmental conditions for disease development, a higher genetic level of resistance to FHB (as expressed by F 201R), could assure concomitantly lower FHB symptoms and a lower accumulation of DON, as compared to susceptible varieties analyzed.

In the case of environment characterized by a consistent mycotoxin synthesis (2006), DON content correlated significantly with phenotypic criteria of assessment, irrespective of the level of FHB resistance expressed by the analyzed varieties (Table 3).

Table 2. Phenotypic FHB traits and DON content of three varieties under artificial inoculation with *Fusarium graminearum* and *F. culmorum* isolates (mean values)

Variety	Phenotypic FHB traits						DON, ppm	
	Severity, %		AUDPC		FDK, %			
	2006	2007	2006	2007	2006	2007	2006	2007
F 201 R	24.7	35.5	230.6	355.5	14.7	21.1	6.2	0.9
Dropia	59.8	61.1	514.4	493.1	33.0	33.0	313.2	0.5
F 133	45.6	69.6	428.2	582.0	39.8	38.0	403.6	1.2
Average	43.4	55.4	391.1	476.9	29.2	30.7	241.0	0.9

Table 3. Correlation analysis of phenotypic FHB traits and DON contamination of grains in three wheat genotypes under artificial inoculation with *Fusarium graminearum* and *F. culmorum* isolates (mean values, 2006)

	Fundulea 201R (Medium resistant)				Dropia (susceptible)				Fundulea 133 (very susceptible)			
	SEV %	AUDPC	FDK %	DON ppm	SEV %	AUDPC	FDK %	DON ppm	SEV %	AUDPC	FDK %	DON ppm
SEV, %	1	0.86*	0.85*	0.86*	1	0.90**	0.94**	0.98***	1	0.98***	0.91**	0.96***
AUDPC	-	1	0.94**	0.75*	-	1	0.97***	0.87**	-	1	0.89**	0.97***
FDK, %	-	-	1	0.91**	-	-	1	0.91	-	-	1	0.86*

Coefficients of correlation calculated evidentiate FDK, % as a potential good indicator for DON accumulation (r = 0.86* to 0.91**). These results are in good agreement with previous reports suggesting the close association between percentage of *Fusarium* diseased kernels (FDK, %) and DON content (Bai et al., 2001). In susceptible varieties, severity and AUDPC correlated better with DON content (r = 0.98*** in Dropia, and r = 0.97*** in Fundulea 133, respectively).

CONCLUSIONS

FHB became in past decades a major threat to food safety and wheat production, because of associated accumulation of fusariotoxins in grains, caused by FHB attack. Experiments conducted at NARDI Fundulea aimed to evaluate factors involved in wheat DON contamination, under artificial inoculation.

Quantification of DON content in wheat varieties sampled from eight locations across Romania, under natural conditions, revealed differences between locations, years and genotype/environment interactions. Efficiency of a higher level of FHB resistance vs. DON contamination under artificial inoculation was demonstrated. In some environments, phenotypic traits, mainly percentage of FDK could be a good indicator of associated contamination of grains with DON.

While the goal of FHB resistance has been met for phenotypic traits, further efforts are required to build by molecular breeding, genotypes combining the both traits, a low content of DON correlated with low FDK, %.

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