AN. I.N.C.D.A. FUNDULEA, VOL. LXXXI, 2013

Electronic ISSN 2067–7758

GENETICA ŞI AMELIORAREA PLANTELOR

www.incda-fundulea.ro

PRELIMINARY EVALUATION OF RESPONSE TO FUSARIUM HEAD BLIGHT (FHB) AND DEOXYNIVALENOL (DON) CONTAMINATION IN SOME ROMANIAN WHEAT AND TRITICALE GENOTYPES

EVALUAREA PRELIMINARĂ A REACȚIEI LA FUZARIOZA SPICULUI ŞI CONTAMINAREA CU DEOXYNIVALENOL (DON) LA UNELE GENOTIPURI ROMÂNEŞTI DE GRÂU ŞI TRITICALE

MARIANA ITTU[1], GHEORGHE ITTU[1], VALERIA GAGIU[2], IRINA SMEU[2] ŞI ALINA DOBRE[2]

Abstract

Fusarium head blight (FHB, scab) is globally a very destructive disease of wheat, triticale and other cereals that drastically could reduce the amount and quality of yields when mycotoxin contamination of grains is associated to the attack. The most common mycotoxin of trichotecenes group is deoxynivalenol (DON), that has a harmful effect on the health of consumers of contaminated food and/or feed and represents a major risk on the food and feed safety. As a consequence, irrespective of use, for feed or/and industry, grains free of hazardous mycotoxin contaminations are demanded. Similarly to other cereals, cultivation of *Fusarium* resistant triticale cultivars is a most promising strategy aimed to achieve this goal. According to the continuous increase of acreage cultivated with triticale in Romania this breeding objective became of major concern at NARDI Fundulea. In this paper are presented preliminary results of evaluating the response to artificial inoculations with *Fusarium culmorum* over three years (2010-2012) and quantification of DON under multienvironment natural conditions (seven locations, 2012), in 13 genotypes of wheat and, respectively 12 of triticale. Results obtained revealed higher values of FHB symptoms on average over three years, expressed in wheat (AUDPC=481), as compared to triticale (AUDPC=111), while a three time higher DON content has been identified in grains of triticale (0.392 ppm), than in wheat (0.137 ppm), on average over seven locations in 2012. Although the maximum level of contamination accepted in U.E. (DON=1.25 ppm) has not been exceeded in any genotype/location combination, a trend of a higher contamination with DON has been observed mainly in locations Secuieni and Targu Mures, where such risks are recommended to be monitored with priority.

Key words: *Triticum aestivum*, *xTriticosecale,* Fusarium head blight, deoxynivalenol (DON).

[1] Institutul Național de Cercetare-Dezvoltare Agricolã Fundulea. E-mail: ittum@ricic.ro

[2] Institutul de Bioresurse Alimentare, Bucureşti.

REZUMAT

Fuzarioza spicelor este o boală foarte păgubitoare răspândită la grâu, triticale şi alte cereale, în toate zonele de cultură din lume. Pe langă reducerea semnificativă a producţiei, acumularea în seminţe a toxinelor specifice (trichotecene), asociate atacului patogenului *Fusarium*, depreciază ireversibil calitatea acestora. Principala toxină din acest grup de micotoxine este deoxynivalenolul (DON), al cărui efect dăunător asupra sănătăţii consumatorilor de alimente sau furaje contaminate reprezintă un serios factor de risc în raport cu siguranţa alimentară. În consecinţă, indiferent de modul de utilizare, pentru consum direct sau prelucrare, sunt necesare seminţe libere de micotoxine dăunătoare. Similar cu alte cereale, pentru atingerea acestui deziderat, cultivarea soiurilor rezistente de triticale si de grâu reprezintă cea mai bună abordare strategică.

Avand în vedere creşterea continuă a suprafeţei cultivate cu triticale în România, acest obiectiv a devenit prioritar în cadrul programului de ameliorare care se desfaşoară la I.N.C.D.A. Fundulea pentru această specie.

In această lucrare sunt prezentate rezultatele preliminare ale evaluării reacţiei la inocularea artificială cu *Fusarium culmorum* (2010-2012) şi analizarea conţinutului de DON în condiţii naturale (şapte localităţi în anul 2012) la 13 genotipuri de grâu şi, respectiv, 12 de triticale.

Rezultatele obţinute au evidenţiat un nivel de patru ori mai ridicat de atac, la genotipurile de grâu, comparativ cu cele de triticale, în medie pe trei ani. În contrast, conţinutul de DON (ppm*)* în probele de seminţe de grâu şi triticale, prelevate în anul 2012 în condiţii de infecţie naturală, din şapte localităţi, a fost în medie de trei ori mai mare la triticale, faţă de grâu.

Trebuie subliniat cã nivelul de contaminare cu DON identificat în anul 2012 la triticale nu a reflectat intensitatea atacului apreciată în aceleaşi condiţii. Deşi, la niciuna dintre combinaţiile genotip/localitate nu s-a depăşit limita de contaminare cu DON admisă în U.E. (DON=1.25 ppm*),* s-a evidentiat, totuşi, o tendinţă mai mare de acumulare a acestei micotoxine în probele de seminţe prelevate din localităţile Secuieni şi Tg. Mureş, fapt care impune, cu prioritate pentru respectivele zone, o monitorizare permanentă a acestui tip de risc.

Cuvinte cheie: *Triticum aestivum*, *xTriticosecale,* Fusarium head blight, deoxynivalenol (DON).

INTRODUCTION

Triticale (*xTriticosecale*) is an intergeneric hybrid between wheat (*Triticum aestivum*) and rye (*Secale crereale*), now grown commercially mostly in Europe, China and Australia, mainly for livestock feed grain or grazing **(**McIntosh, 1988) and bioethanol.

Triticale is considered as a valuable crop because of its yield ability adapted to less favourable soil conditions and low input farming. Disease resistance is particularly important for farming with limited or no use of pesticides (e.g., organic farming). Simultaneously, triticale is a good resource of genes for wheat improvement, that it is used as a bridge to transfer desirable characteristics of rye into wheat (Qualset et al., 1985; Zhuang and Li, 1993; Saulescu et al., 2010), such resistance to wheat stem rust (Adhikari and McIntosh, 1998; Singh and McIntosh, 1988; Zhang et al., 2010), induced by several resistance genes such *Sr* 27, *srNin, SrSatu, SrBj* and *SrVen* (McIntosh et al., 1995). Assessment of seedling reaction to races of *P. graminis* f. sp. *tritici* with broad virulence, including the recently emerged TTKSK, TRTTF and TTTTF (Pretorius et al., 2000) in a collection of 567 accessions originating from 21 countries, revealed the presence of uncharacterized genes in 129 (79.6%) of them (Olivera et al., 2013).

Although triticale inherited from rye a natural resistance to diseases, as a consequence of broader and more intensive cultivation, new and specialized pathogens have appeared and similarly to other species, have broken its initial resistance. This challenge regarding a relatively new crop emphasizes the need to permanently monitor the occurrence of

potentially damaging diseases and pests during the growing period, in order to properly manage the risks by reliable control methods. (Anonymous: Thüringer Landesanstalt für Landwirtschaft).

In the last years, more frequent and intense leaf rust and powdery mildew attacks have been observed in triticale, studies on a possible specialization of *Puccinia* versus this new plant host being under progress (Hanzalova and Bartos, 2011). Currently, the response of triticale to Fusarium head blight, particularly to the contamination of grain with deoxynivalenol (DON), is of outmost importance in many research programs across the world, because of its harmful effect of the health of consumers and consequently on *food* and *feed safety*. DON is the main mycotoxin from the trichotecene group associated to *Fusarium* infection that determines the fungal spread and disease development in *Triticeae* (Langevin et al., 2004).

Irrespective of use, for feed or/and industry, grains free of hazardous mycotoxin contaminations are demanded. In this context, the cultivation of *Fusarium* resistant cultivars, including triticale, is a most promising strategy for avoiding toxin problems, although breeding of resistant cultivars has been hampered up to now by the lack of efficient resistance sources and tedious testing for improved FHB resistance. Reducing of mycotoxin content in grain of triticale according to FHB rating of spikes is more difficult as far as the both traits are not tightly associated as compared to wheat. Analysis of 113 and 55 triticale genotypes for symptom development on spikes under multienvironment artificial inoculation, *Fusarium* exoantigen (ExAg) and quantification of DON content by immunoassay revealed a higher correlation in wheat (r=0.86) than in triticale (r=0.60) (Miedaner et al., 2004). These findings suggesting different patterns of DON contamination in wheat and triticale, were confirmed by independent comparative studies performed in Poland with 32 winter triticale cultivars and 36 of winter wheat ones (Goral et al., 2013).

In order to reduce the mycotoxin content in triticale grain, very promising seems to be the use of a novel and highly resistant source line as donor of FHB resistance and a quantitative trait loci (QTL) mapping scheme for the identification of chromosomal segments harbouring FHB resistance genes initiated since 2012 at IFA-Tulln, Austria, focused on reducing the risk of mycotoxin contamination in triticale. (Research Project *Smart Breeding of Low Mycotoxin Triticale, 2012-2015, https*://forschung.boku.ac.at/fis/suchen.projekt_uebersicht?sprache_in=en&menue_id_in=300&id_in=9577 r).

In Romania, triticale is grown, mainly, in the hilly regions on the acid poor fertile soils and covers, yearly, around 100-130 thousands ha or 1.5% from the arable land. Since 1971, when the breeding program has been started, up to present, it has been developed an adapted triticale germplasm for the Romanian environmental conditions and 12 new varieties have been registered. Genetic progress for yield, estimated over a 27 years, is by 46 kg ha^{1} year^{1} or 0.80% year^{1}, similarly with those realized in the most dynamic triticale breeding programs of the world. The improving of yields has been achieved by an increased number of kernels per spikes, plumpness of kernels, test weight and reduction of the plant height by introduction in the Romanian triticale germplasm of *RhtB1b* (*Rht1*)

and *Ddw1* (*Hl*) genes. The genetic gain for reduction of plant height, in this period, was estimated at 1.16 cm yr^{1}.

Further progress regarding yield stability under conditions of global climatic changes, a broader genetic diversity for preharvest sprouting (PHS), drought tolerance, earliness, high canopy albedou, diseases resistance, especially for Fusarium head blight (low DON content in kernels), leaf rust and BYDV, is required. With regard to *Fusarium* this demand is empaphasized by the possible shift to an improved toxicity of the pathogen in Romania. Molecular analysis revealed the prevalence of 15-AcetylDON (15ADON) chemotype, in isolates sampled from wheat and triticale, but the co-occurrence of some isolates of chemotype 3-AcetylDON (3ADON), characterized by a higher toxicity, in some local *Fusarium* populations has been also found (Ittu et al., 2012).

In the last decades, important progress has been obtained in the triticale breeding programs worldwide, but consequently to the increasing of grown area, crop became more vulnerable, than before, to diseases, manly to rusts, powdery mildew etc (Ittu et al., 2008).

The aim of this paper is to evaluate comparatively the response to artificial inoculations with *Fusarium* and quantification of DON in wheat and triticale (in natural inoculation in seven locations).

MATERIAL AND METHODS

Plant host. In this study were tested under artificial and natural conditions, 13 genotypes of wheat and, respectively 12 of triticale, representative for the presently released varieties from Romania. A standard sample of 200 g from each variety of wheat and triticale has been obtained from seven locations across Romania where these crops are usually grown: Albota, Şimnic (South-West), Tg. Mureş, Turda (North), Livada (North-West), Valu lui Traian (South-East) and Secuieni (North-East).

Inoculation. Under field conditions from three environments (years 2010, 2011 and 2012) 10 spikes/entry were inoculated in the FHB experimental field at Fundulea, with a very aggressive isolate of *Fusarium graminearum* at anthesis, by point (single) method.

Assessment of resistance. Response to *Fusarium* infection was assessed by counting the damaged spikelets at 20 days post inoculation (d.p.i.) and computed as area under disease progress curve (AUDPC).

DON quantification. DON content of all samples was analyzed at the Microbiology unit from the Institute of Food Bioresources, Bucharest, by a commercially available enzyme immunoassay (RIDASCREENTM FAST DON, R-Biopharm GmbH, Darmstadt, Germany), upon the producer's recommendations, being expressed in ppm.

RESULTS

The values computed for AUDPC evaluated under artificial inoculation over three years, were on average four time higher in wheat (AUDPC=481.0), as compared to those registered in triticale (AUDPC=111.0) (Table 1). In contrast with symptoms, the overall grain content of DON, has been in all locations investigated in 2012, three times higher in

triticale (mean values DON=0.392 ppm), than in wheat (mean values DON=0.137 ppm), while the avoided limit of contamination in E.U. (0.125 ppm), has not been exceeded in any genotype by location combination (Tables 2).

However, the level of contamination with DON found in triticale, relatively low has not been strongly associated to the intensity of *Fusarium* attack, found in 2012 under natural conditions (data not shown) and under artificial inoculation.

Table 1

Response to Fusarium head blight under artificial inoculation, expressed as AUDPC, in 13 genotypes of wheat and 12 genotypes of triticale
(Reacția la fuzarioza spicului, exprimată pe baza AUDPC, la 13 genotipuri de grâu şi 12 de triticale), Fundulea, 2010-2012

No. (Nr. crt.)	WHEAT (Grâu)		No.	TRITICALE (Triticale)	
	Entry (Varianta)	AUDPC (mean values across 3yrs) (valori medii, trei ani)		Entry (Varianta)	AUDPC (mean values across 3yrs) (valori medii, trei ani)
1	Dropia	500	1	Plai	138
2	Boema1	468	2	Titan	94
3	Delabrad 2	472	3	Stil	94
4	Faur-F	427	4	Gorun1	84
5	Glosa	481	5	Haiduc	92
6	Izvor	518	6	Cascador F	149
7	Litera	577	7	Mezin	109
8	Otilia	461	8	Negoiu	133
9	Ostrov	312	9	Oda	85
10	66475G1-2	454	10	Paltin	87
11	06476G5-3	503	11	Pisc	103
12	Pitar	575	12	Rotric	168
13	Partener	500			
Mean		481			111
LSD 5% *LSD 1%* *LSD 0.1%*		*219.9*			*39.4* *53.7* *72.1*

Table 2

Content of DON in grain of wheat and triticale under natural conditions (mean values over seven locations, 2012)
(Conținutul de DON în semințe la grâu şi triticale, în condiții naturale - valori medii, sapte localități, 2012)

No. (Nr. crt.)	DON content, ppm				
	WHEAT (Grâu)		No.	TRITICALE (Triticale)	
	Entry (Varianta)	Mean values over seven locations (Valori medii/ şapte localități)		Entry (Varianta)	Mean values over seven locations (Valori medii/ şapte localiăți)
1	Dropia	0.192	1	Plai	0.148
2	Boema1	0.139	2	Titan	0.329
3	Delabrad 2	0.186	3	Stil	0.248
4	Faur-F	0.116	4	Gorun1	0.271
5	Glosa	0.089	5	Haiduc	0.555
6	Izvor	0.169	6	Cascador F	0.508
7	Litera	0.132	7	Mezin	0.437
8	Otilia	0.078	8	Negoiu	0.552
9	Ostrov	0.117	9	Oda	0.328
10	66475G1-2	0.222	10	Paltin	0.362
11	06476G5-3	0.097	11	Pisc	0.543
12	Pitar	0.152	12	Rotric	0.427
13	Partener	0.095		-	-
Mean		***0.137***			***0.392***
LSD 5% *LSD 1%* *LSD 0.1%*		*0.083* *0.110* *0.143*			*0.200* *0.260* *0.336*

Data obtained in this investigation revealed significant differences between the analysed genotypes of wheat and triticale, regarding the FHB symptoms (AUDPC) and grain contamination with DON (ppm). Analysis of relation between the overall DON content in grain of either of the both crops according to the origin of samples (seven locations), suggests a higher degree of contamination with DON in samples from Secuieni and Tg. Mures, irrespective of the plant host.

These findings confirm previous observations corresponding to the same locations (*data not shown*), but the same tendency seems to be true also for other regions such: Livada (wheat) and Albota, Livada (triticale) that could be considered as potential hot spots in terms of DON contamination (Figure 1).

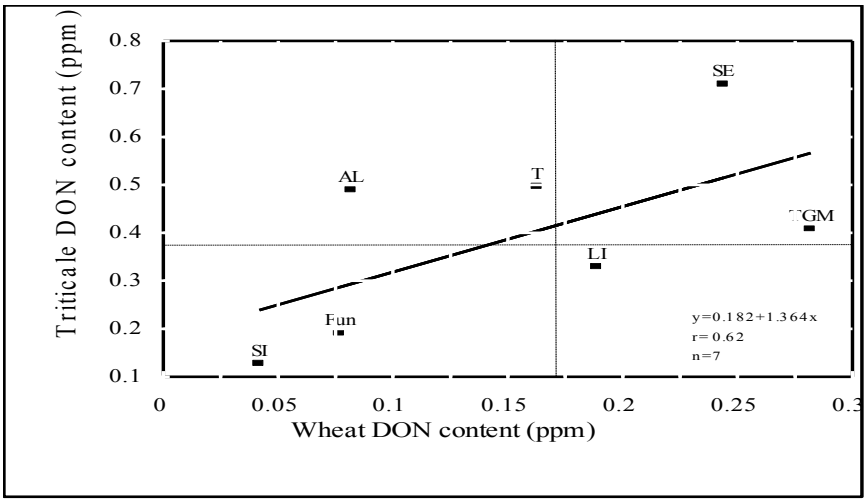

Figure 1. Relation between the contamination with DON (ppm) in grain of triticale and wheat (mean values over seven locations, 2012)
[Relația dintre contaminarea cu DON (ppm) în semințe de grâu şi triticale (valori medii, sapte localități, 2012)]

CONCLUSIONS

Significant genotypic differences regarding the FHB symptoms (AUDPC) and DON contamination (ppm) were found in the investigated genotypes of wheat and triticale;

Contrasting response to *Fusarium* attack and contamination with associated toxin DON was found in triticale, as compared to wheat, the expression of symptoms being much lower (four times than in wheat) related to the amount of DON resulted from analysis (three times higher in triticale).

Acknowledgements

Wheat and triticale samples analysed in this report have been provided by the courtesy of Dr. Maria VOICA (ARDS Albota), Dr. Gabriela PAUNESCU (ARDS Şimnic), Eng. Iustina LOBONȚIU (RDSCB Tg. Mureş), Dr. Vasile MOLDOVAN (ARDS Turda), Dr. Cecilia BĂNĂȚEANU (ARDS Livada), Dr. Ioana STERE (ARDS Valu lui Traian) and Eng. Simona Pochişcanu (ARDS Secuieni).

REFERENCES

ADHIKARI, K. N., AND MCINTOSH, R. A., 1998. *Inheritance of wheat stem rust resistance in triticale*. Plant Breed., 117: 505-513.

GÓRAL, T., WIŚNIEWSKA, H., OCHODZKI, P., WALENTYN-GÓRAL, D., GRZESZCZAK, I., BELETR, J., BANASZAK, Z., POJMAJ, M., KURLETO, D., KONIECZNY, M., BUDZIANOWSKI, G., CICHA, A., PAIZERT, K., WOŚ, H., 2013. *Variation for Fusarium head blight resistance and Fusarium toxins accumulation in winter triticale lines inoculated with Fusarium*

culmorum. Book of Abstracts of the 12th European Fusarium Seminar, 12-16 May 2013, Bordeaux, France: 181.

HANZALOVÁ, A. AND BARTOŠ, P., 2011. *Resistance of Triticale to Wheat Leaf Rust (Puccinia triticina*). Czech J. Genet. Plant Breed., 47, 2011 (1): 10-16.

ITTU, M. AND ITTU, G., 2008. *Latest in breeding of resistance to FHB in Romanian triticale*. Cereal Research Communications, 36, Suppl. B: 3: 103-105.

ITTU, M., CANA, L., CIUCA, M., VOAIDES, C., AND CORNEA, P., 2012. *Phenotypic and marker assisted evaluation of aggressiveness toward wheat in some Romanian Fusarium populations*. Romanian Agricultural Research, 29: 289-296.

LANGEVIN, F., EUDES, F. AND COMEAU, A., 2004. *Effect of Trichothecenes produced by Fusarium graminearum during Fusarium head blight development in six cereal species.* European Journal of Plant Pathology, 110: 735-746.

MCINTOSH, R. A., 1988. *The role of specific genes in breeding for durable stem rust resistance in wheat and triticale.* In: Breeding Strategies for Resistance to the Rusts of Wheat. Pages 1-9, CIMMYT, Mexico D.F., Mexico. Genes. Kluwer Academy Publishers, London.

MIEDANER, T., HEINRICH, N., SCHNEIDER, B., OETTLER, G., ROHDE, S., RABENSTEIN, F., 2004. *Estimation of deoxynivalenol (DON) content by symptom rating and exoantigen content for resistance selection in wheat and triticale*. Euphytica, *139*: 123-132.

PRETORIUS, Z. A., SINGH, R. P., WAGOIRE, W. W., AND PAYNE, T. S., 2000. *Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis f. sp. tritici in Uganda*. Plant Dis., 84: 2003.

QUALSET, C. O., 1985. *Synthesis, breeding, adaptation, and utilization of triticale in California*. Pages 71-82. *In:* Genetics and Breeding of Triticale. M. Bernard and S. Bernard (eds). Proc. 3rd EUCARPIA Meet. Triticale, Clermont-Ferrand, France.

SAULESCU, N.N., ITTU, G., CIUCA M., ITTU, M., SERBAN, G., AND MUSTATEA, P., 2011. *Transferring useful rye genes to wheat, using Triticale as a bridge*. Czech Journal of Genetics and Plant Breeding, 47: 556-562.

SINGH, S. J., AND MCINTOSH, R. A., 1988. *Allelism of two genes for stem rust*. Euphytica, 38: 185-189.

ZHANG, J., WELLINGS, C. R., MCINTOSH, R. A., AND PARK, R. F., 2010. *Seedling resistance to rust diseases in international triticale germplasm.* Crop Pasture Sci., 61:1036-1048.

ZHUANG, Q. S., AND LI, Z. S., 1993. *Present status of wheat breeding and related genetic study in China.* Wheat Inf. Serv., 76:1-15.

Prezentată Comitetului de redacție la 8 noiembrie 2013