

DOI: 10.2478/v10129-011-0018-x

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PHENOTYPIC AND MARKER ASSISTED EVALUATION
FOR PATHOGENICITY AND AGGRESSIVENESS
OF ROMANIAN *FUSARIUM* ISOLATES VS. WHEAT

ABSTRACT

Pathogenicity and aggressiveness vs. wheat of 30 new *Fusarium* accessions, primarily *F. graminearum*, obtained from random naturally infected grain samples of bread wheat, durum wheat and triticale collected across Romania, phenotypically and by molecular tools has been investigated. A large variation of this trait, expressed as reduction of coleoptiles length (% of control), in seedling stage, on average over three varieties, ranging from 2.1 to 30.9 % was registered. Point field inoculations at anthesis of 90 *Fusarium* isolate x wheat varieties combinations, revealed also the variability of several components of aggressiveness: *severity* (14.4-64.8%), *AUDPC* (104.9-527.1), and *FDK* 8.1-43.7%, respectively.

Molecular techniques allowed identification of *Fusarium* species and the analysis of polymorphism within fungal isolates. Moreover, the presence of *TRI 5* gene involved in DON biosynthesis was detected in the majority of isolates.

Similarity between records obtained in seedling and adult stage for the most aggressive of *Fusarium* isolates, suggests that phenotypic selection in conjunction with molecular tools could be a reliable method to select the appropriate pathogen strains for breeding of resistance.

Key words: aggressiveness, *Fusarium graminearum*, *F. culmorum*, molecular polymorphism, TRI 5 gene

INTRTODUCTION

Fusarium graminearum [(teleomorph *Gibberella zeae* (Schwein.) Petch.] and *F. culmorum* are known as main producers of fusarium head blight, FHB, disease that threatens wheat production across the world and poses

a significant public health hazard, because of association with mycotoxin accumulation in grains (Pestska and Smolinski, 2005). Deoxynivalenol (DON, vomitoxin) with its analogs, 15-ADON and 3-ADON, is one of the most common trichothecene toxin produced by *F. graminearum*, representing an aggressiveness factor on wheat (Bai *et al.*, 2002). Large genetic variability in *F. graminearum* with close relation among different lineages (*phylogenetic species*) was reported (Bowden and Leslie, 1999) and a QTL for aggressiveness linked to the *TRI 5* locus was detected (Cumagun *et al.*, 2004).

Deployment of resistant germplasm is the most accepted component of the strategy to control FHB in spite of difficulties to achieve this goal (Miedaner *et al.*; 2001; Bai and Shaner, 2004). Resistance of wheat to FHB, regardless components already described (Schroeder and Christensen, 1963), is multigenic, quantitative, where no immune cultivar and clear host by species interaction are known (Van Eeuwijk *et al.*, 1995, Bai and Shaner, 1996). Recently a shift in *Fusarium* population from North America toward an increased frequency and aggressiveness, of the newly emerging 3ADON, as compared to the previously prevalent 15 ADON, have been reported (Ward, *et al.*, 2008). Marker assisted selection (MAS) for host resistance and pathogen aggressiveness were employed in the past years in order to a better understanding and validation of these traits in *Fusarium*/wheat pathosystem. QTL associated with FHB resistance, Type I and Type II and more recently with DON detoxification (Type III *sensu* Miller and Arniston, 1986) were identified on almost all of the wheat chromosomes (Buerstmayr *et al.*, 2009; Liu *et al.*, 2009; Zhang *et al.*, 2010). PCR markers for trichothecene genes (*TRI*) expression of *Fusarium graminearum* in order to predict occurrence of new chemotypes of *F. graminearum* and new techniques aimed to identify resistance to FHB in wheat were developed (Chandler *et al.*, 2003; Gosman *et al.*, 2010). That's why basic information about pathogenic ability in order to maximize the breeding efforts toward the management of FHB risks is needed.

Our objectives were:

- i. to estimate the phenotypic variation of aggressiveness in *Fusarium* isolates obtained from several local populations of pathogen under artificial inoculation in seedling and adult stages,
- ii. identification of *Fusarium* species with PCR markers, and
- iii. to elucidate the polymorphism of *Fusarium* isolates.

Fusarium isolates analyzed for pathogenicity and aggressiveness under artificial inoculation in seedling and adult stage

Table 1

Fusarium code	Origin			Stage	
	Year	Location	Host	Seedling	Adult
F. g. 983, F.g. 991	2008	Brasov	durum	X	-
F. c. 1056	2008	Simnic	wheat	X	X
F. g. 1145	2008	Albota	wheat	X	X
F. g. 1137, F.g. 1143, F. g. 1156, F. g. 1182	2008	Albota	wheat	X	-
F. g. 1169	2008	Albota	triticale	X	-
F. g. 1204, F.g. 1211	2008	Tg.Mures	wheat	X	X
F. g. 1224, F. g. 1228, F. g. 1237, F. g. 1239, F. g. 1265, F. g. 1272	2008	Livada	wheat	X	X
F. g. 1216, F. g. 1220, F. g. 1226, F. g. 1274, F. g. 1278, F. g. 1222, F. g. 1238, F. g. 1266	2008	Livada	wheat	X	-
F. g. 1313, F. g. 1316, F. g. 1317, F. g. 1318, F. g. 1343	2008	Livada	triticale	X	-

MATERIAL AND METHODS

Pathogen

Thirty *Fusarium* accessions obtained at NARDI-Fundulea from the naturally infected grains of bread wheat, durum wheat and triticale, randomly sampled in 2008 from five locations (Albota, Livada, Brasov, Simnic and Tg. Mures) were analyzed in seedling stage, while for field experiments by point inoculation only ten selected *Fusarium* isolates of them were used (Table 1). Homogenized suspensions of conidia in distilled water (about 500000/ml), produced from each of *Fusarium* cultures were used for artificial inoculation.

Plant material

Nine adapted Romanian varieties (Mustatea *et al.*, 2009) released between 1991-2004, by NARDI Fundulea (Dropia, Boema, Dor, Delabrad) and the wheat breeding centers from Turda (*Ariesan*, *Apullum*, *Dumbrava*), Pitesti (*Trivale*), and Simnic (*Briana*), were inoculated at anthesis by point artificial inoculations in the FHB experimental field at Fundulea.

Pathogenicity and aggressiveness test. In seedling stage, aggressiveness of *Fusarium* isolates *vs.* three wheat entries according to the protocol established by Ittu (1986) was evaluated. Pathogenic potential of *Fusarium* isolates was expressed as reduction of coleoptiles length in eight days old seedlings. In adult stage aggressiveness was analyzed in 90 host x pathogen combinations, in terms of *severity* (damaged florets at 20 days post inoculation, %), *area under disease progress curve* (AUDPC) and Fusarium damaged kernels, FDK %.

Genotyping.

DNA isolation from each fungal isolate was performed by the method described by Stepien *et al.*, 2008. Three pair primers for the identification of *F.graminearum* (Fg16F/Fg16R), *F.culmorum* (FC01F/FC01R) and *S.sporotrichoides* (Fspo1F/Fspo1R) were used in a multiplex PCR reaction according to Demeke *et al.* (2005). Molecular polymorphism of the fungal isolates was analyzed by RAPD technique, using seven arbitrary primers (OPA17, OPA19, OPC06, OPG06, OPR05, UBC147, UBC180 and UBC186) from Operon Technology and University of British Columbia, respectively. The presence of *TRI 5* gene was checked in a PCR reaction using *tox5-1/tox5-2* primer pair (Niessen and Vogel, 1998). The PCR products were analyzed by electrophoresis on 1.5% agarose gel in 0.5 ×TBE (Sigma-Aldrich) stained with 0.3µg/ml ethidium bromide. Pictures from electrophoresis gels have provided the information for phylogenetic tree. Each band was considered as a locus (presence of the band was scored as 1

and its absence as 0). All bands were studied except weak and incomplete ones. Genetic distance was computed by Nei & Li (1979) formula, using TREECON 1.3b software package. Dendrogram was prepared by UPGMA (Unweighted Pair Group Method with Arithmetical Averages).

Statistical analysis.

Analysis of variance (ANOVA) was used to estimate the contributions attributable to genotypes of the pathogen and host.

RESULTS & DISCUSSION

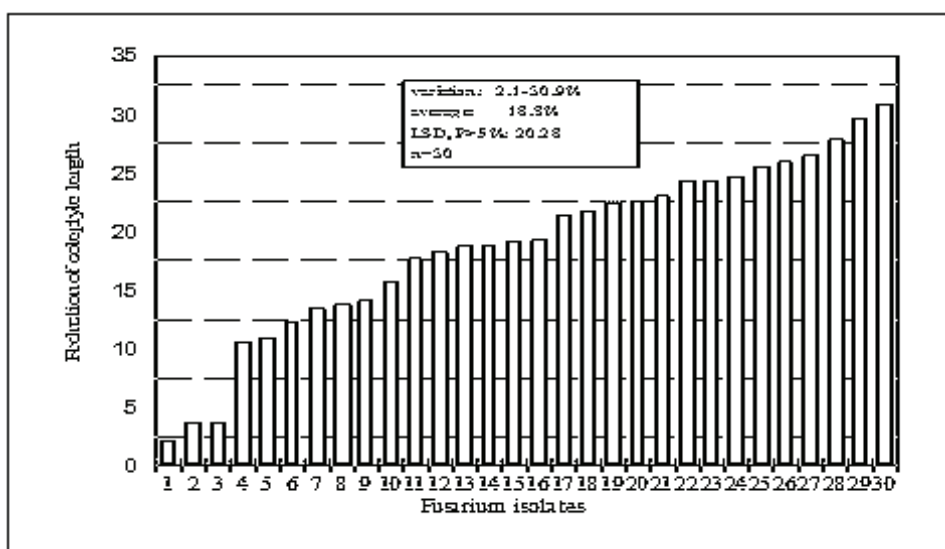


Fig. 1. Estimation of *Fusarium* aggressiveness in seedling stage (Fundulea, 30 isolates, mean values over three wheat cultivars).

Fusarium isolates:

- | | | | |
|------------------|--------------------|------------------|------------------|
| 1)l. F.g. 1266, | 2)l. F.g. 1216, | 3)l. F.g. 1143, | 4)l. F.g. 1313; |
| 5)l. F.g. 983, | 6)l. F.g. 1220, | 7)l. F.g. 1169, | 8)l. F.g. 1318, |
| 9)l. F.g. 991, | 10)l. F.g. 1222, | 11)l. F.g. 1137, | 12)l. F.g.1237, |
| 13)l. F.g. 1278, | 14)l. F.g. 1238, | 15)l. F.g. 1317, | 16)l. F.g. 1316, |
| 17)l. F.g. 1343, | 18)l. F.g. 1224, | 19)l. F.g. 1211, | 20)l. F.g. 1274, |
| 21)l. F.g. 1145, | 22)l. F.g. 1239, | 23)l. F.g. 1204, | 24)l. F.g. 1265, |
| 25)l. F.g. 1156, | 26)l. F.g. 1182, | 27)l. F.g. 1272, | 28)l. F.g. 1226, |
| 29)l. F.g. 1228, | 30)l. F.c. 1056)l. | | |

A broad range of aggressiveness variation among *Fusarium* accessions in seedling stage was found. Relatively highly aggressive *Fusarium* isolates that produced a reduction of coleoptiles length exceeding 25.0% as compared with control, were obtained from naturally infected samples originated from Albota (*F.g. 1156*, *F.g. 1182*), Livada (*F.g. 1272*, *F.g. 1226*, *F.g.*

1228) and Simnic (*F.c. 1056*) (Fig. 1). In adult stage a large variation of aggressiveness in terms of severity (%), AUDPC and FDK (%) was found (Table 2). A good agreement between records obtained in the both stages of wheat development, particularly for *Fusarium* isolates, *F.c. 1056*, *F.g. 1272* and *F.g. 1228* was observed.

Table 2

Components of aggressiveness under field point inoculation (Fundulea 2009, ten isolates vs. nine wheat varieties, mean values)

Entry	Severity [%]			AUDPC			FDK [%]		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
F. c. 1056	28.3	92.4	61.8	217.5	707.0	527.1	10.4	82.3	43.7
F. g. 1265	11.4	75.0	42.8	89.5	536.0	312.0	1.9	59.1	30.5
F. g. 1224	31.6	100.0	64.8	231.0	800.0	489.0	0.8	55.0	29.0
F. g. 1272	15.1	65.7	44.3	145.5	462.5	321.3	0.7	46.5	27.5
F. g. 1237	2.6	68.5	48.9	16.0	464.5	329.6	0.0	41.4	26.6
F. g. 1145	20.8	62.7	46.9	193.0	458.5	342.0	8.6	49.0	26.0
F. g. 1211	22.6	79.7	54.1	159.0	753.0	439.9	3.5	57.0	26.0
F. g. 1204	16.5	57.3	39.7	113.5	437.0	298.4	3.2	54.6	25.7
F. g. 1228	15.0	82.3	40.7	147.0	593.5	326.6	5.0	38.5	22.0
F. g. 1239	8.3	23.9	14.4	72.0	145.5	104.9	0.0	18.3	8.1
Average	17.2	70.8	45.8	138.4	535.8	349.1	3.41	50.17	26.51
Minimum	2.6	23.9	14.4	16.0	145.5	104.9	0.0	18.3	8.1
Maximum	31.6	100.0	64.8	231.0	800.0	527.1	10.4	82.3	43.7
LSD P<5%			11.9			79.9			9.99

Molecular analysis. Based on the expected amplicons produced by specific primers, among the 30 fungal isolates analysed, 29 belong to *F.graminearum*, while isolate *F.c.1056* was identified as *F.culmorum* (*data not shown*). RAPD analysis have suggested a relative reduced molecular polymorphism of analyzed *Fusarium* isolates, distinct differences being observed in some of the isolates especially with OPA 19 primer. Based on the electrophoretic pattern of the amplicons, two clusters of strains with TREECON program were detected: one including the isolates *F.g.1228*, *F.g.1265*, *F.g.1182* and *F.g.1137*, while the others are grouped in the second one which contains also several sub-clusters (Fig.2). Using the primer set *tox5-1/tox5-2*, specific for presence of *TRI 5* gene, the corresponding amplicon suggesting their ability to produce DON was found in all *Fusarium* isolates tested, excepting *F. g. 1266* and *F.g.1216* (Fig.3).

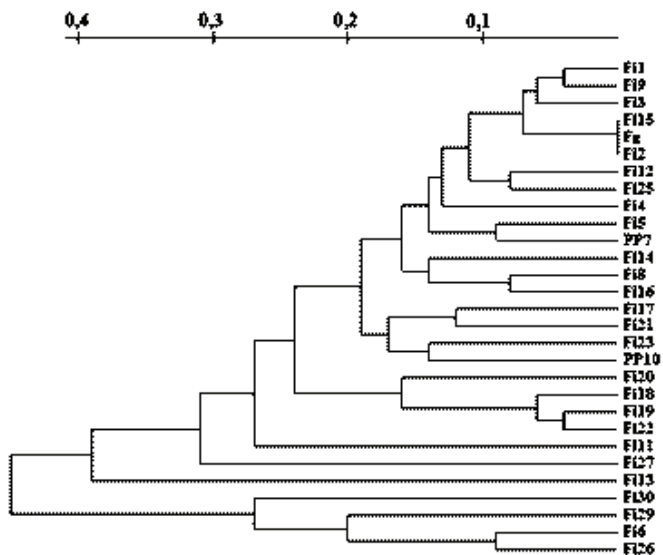


Fig.2. Dendrogram resulted after RAPD analysis of Romanian

Fusarium isolates.

- | | | | |
|-----------------|-----------------|-----------------|------------------|
| (Fi1-F.g. 983; | Fi9- F.g. 1216; | Fi3-F.g. 1143; | Fi15-F.g. 1278; |
| Fi2-F.g. 991; | Fi12-F.g. 1226; | Fi25-F.g. 1266; | Fi4-F.g. 1145; |
| Fi5-F.g. 1156; | Fi14-F.g.1274; | Fi8-F.g. 1211; | Fi16-F.g. 1313; |
| Fi17-F.g. 1316; | Fi21-F.g. 1222; | Fi23-F.g. 1272; | Fi 20-F.g. 1343; |
| Fi18-F.g. 1317; | Fi19-F.g. 1318; | Fi22-F.g. 1239; | Fi11-F.g. 1224; |
| Fi27-F.g. 1169; | Fi13-F.g. 1237; | Fi30-F.g. 1228; | Fi29-F.g. 1265; |
| Fi6-F.g. 1182; | Fi26-F.g. 1137) | | |

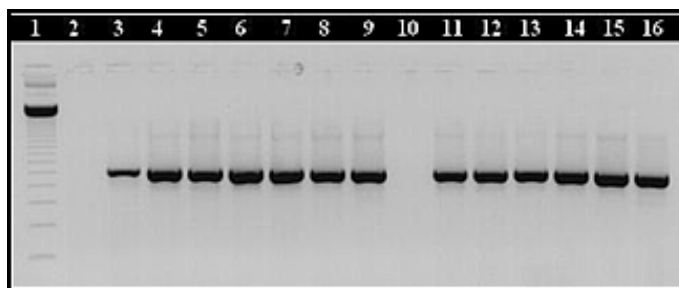


Fig.3. Amplification products obtained by PCR reaction carried out using DNA purified from *Fusarium* isolates and primer set *tox5-1/tox5-2*:

- | | | | |
|---------------------------|----------------|----------------|----------------|
| 1 – ladder 123 bp (Roth); | 2-F.g.1266; | 3-F.g.983; | 4-F.g.991; |
| 5-F.g.1143; | 6-F.g.1145; | 7-F.g.1156; | 8-F.g.1182; |
| 9 - F.g.1211; 9 - | 10 - F.g.1216; | 11 - F.g.1224; | 12 - F.g.1226; |
| 13 - F.g.1237; | 14 - F.g.1274; | 15 - F.g.1278; | 16 - F.g.1313. |

ACKNOWLEDGEMENTS

Research was funded by the grant from the Romanian Ministry of Education and Research-National Center for Program Management (Management of risk of pre-harvest *Fusarium* mycotoxins contamination in wheat, PNCD 2-51040/2007)

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