THE AGGRESSIVENESS OF *FUSARIUM GRAMINEARUM* AND COMPARISON OF ZEARALENONE ACCUMULATION ON WHEAT GENOTYPES

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Abstract

Fusarium graminearum is one of the most important causal agents of Fusarium head blight (FHB), results in yield and quality reductions as well as mycotoxin accumulation in the infected kernels. This research was conducted in Agricultural Research Station in Canakkale Onsekiz Mart University in subsequent two years. In this research the reactions of 15 wheat cultivars to F. graminearum was evalueted in means of disease severity, relative head weight and Zearalenone (ZEA) accumulation. The wheat heads were inoculated by conidium suspension of a virulent isolate of the fungus by a syringe at mid-anthesis stage. Head blight severity was assessed at 21 days after inoculation. Relative head weight of the infected heads was also determined after harvest. In addition, the ZEA contents in infected kernels was also compared. When inoculated with the fungus wheat genotypes exhibited different reactions. The disease severity in different wheat genotypes was differed between 24.7-78.8%. Relative head weight of the heads infected by the fungus was also statistically different. The ability of the isolate for ZEA accumulation was significant among the wheat genotypes. We found a negative correlation between relative head weight and disease severity, but positive correlation between ZEA accumulation and disease severity.

Keywords: *fusarium graminearum, wheat, fusarium head blight, relative head weight, mycotoxin, zearalenone*

1. INTRODUCTION

The fungal pathogen *Fusarium graminearum* (Teleomorph: *Gibberella zeae)*, is one of the most common causal agent of Fusarium head blight (FHB) in wheat growing areas. Although the disease affects wheat; barley and other small grains and maize also hosts the fungus (Dill-Macky, 2010).

FHB is initiated by airborne multicelled conidium on mid-anthesis stage in the fields. The fungus colonizes the tissues spreading upwards and downwards of the head resulting necrosis or bleching in the infested spikelets. The initial symptom at the infection site seems like water-soaked, then the tissue becomes discolered because of the spread of the fungus intracellularly. Premature bleaching of head tissues is a characteristic of the disease in wheat fields (McMullen et al., 1997).

F. graminearum reduce crop quantity by either affecting sterility as the fungus opts to infect the ovarial tisses of hosts. Crop reduction was also observed because of scabby seeds so-called *Fusarium* damaged kernels. The shriveled grain is normally blown away during harvest. The most important effect of FHB is the accumulation of mycotaxins in infected plant tissues. Beasuse mycotoxins have impact on human and animal health, more attention was given to FHB in cereal crops (Bai and Shaner, 1994).

F. graminearum produces several mycotoxins. Most important groups are deoxynivalenol (DON) and derivatives and zearalenone. The most important economic and health concerns of FHB are due to mycotoxin contamination. The *Fusarium* species accumulate three classes of mycotoxins in colonized tissues. Fumonisins is possibly associated with brain damage in horses. The next important group fall into the chemical class called trichothecenes including the best known mycotoxins vomitoxin (deoxynivalenol, or DON) and nivalenol. Zearalenone is the third chemical class of *Fusarium* mycotoxin. It possesses estrogenic activity, therefore it may have a possible role in reproductive system problems of both humans and animals (Bottalico, 1998; Desjardins, 2006).

There are several reports about the differences in aggressiveness among *F. graminearum* isolates sampled from various parts of the world (Bai and Shaner, 1996; Miedaner et al., 2003). Aggressiveness of *F. graminearum* is not restricted to geographically isolted areas, besides there could be a great differeneces in aggressiveness amoung *F. graminearum* isolates even in a small limited areas. Large genetic variation in terms of aggressiveness also exists among these isolates collected from different countries and continents (Miedaner and Schilling,1996; Miedaner et al., 2003).

Similar observations were made when the reactions of wheat genotypes against *F. graminearum* studied. Tunalı et al. (2006) reportad that wheat genotypes exhibited great variation in means of disease severity, a-tousand-kernel weight, relative head weight and DON accumulation when inoculated with *F. graminearum* and *F. culmorum*. Similar results were also observed in other report. (Bai and Shanner, 1996; Miedaner et al., 2003)

The objective of this study was to compare the reactions of 15 wheat genotypes against *F. graminearum* infection in means of disease severity, relative head weight and ZEA accumulation.

2. MATERIAL AND METHODS

2.1. Inoculum Preperation and Inoculation

Isolate of *F. graminearum* was collected from infected wheat heads. Infected grain samples were surface sterilized with 1% sodium hypochlorite solution and rinsed three times with sterile distilled water. After drying in a fume hood to eliminate excess moisture, they were placed in potato dextrose agar (PDA) medium containing 100 µg/mL streptomycin sulfate and incubated at 25^0C for 5-7 days. Monoconidial isolates were obtained by streaking spore suspensions onto water agar plates and single conidia were transferred onto new plates.

The isolate was examined after 10 days and selected based on pigmentation on PDA. It was grown on carnation leaf agar (CLA) and identified as *F. graminearum* by conidial morphology and colony characteristic as described by Leslie and Summerell, 2006. Identification of the species was also proved by PCR-based method using *F. graminearum*-specific primers, Fg16 and Fg16N, as described by Nicholson et al. (1998).

Stored cultures were grown on malt extract agar (MEA) for 2-3 weeks. Then 5 mL of sterile distilled water poured on the grown culture to harvest the conidia (Engle et al., 2003). Conidium suspension was adjusted to $1X10^5$/mL and wheat heads were inoculated at mid-anthesis stage using a micropipette. Approximately 10 µl of inocula was applied to central spikes and covered with transparent plastic bags to maintain humidity for 48 hours.

2.2. Assessment of Disease Severity and Relative Head Weight

Disease severity was assessed by counting the number of visually diseased spikelets 21 days after inoculation and relating them to the total number of spikelets of the respective head, resulting in percent infected spikelets.

Spikes were harvested after maturation to find out relative head weight. Relative head weight was calculated as dividing the weight of infected heads to the weight of uninfected control heads.

2.3. Zearalenone Quantification

ZEA content was extracted from infected and uninfected kernels. The method was briefly based on extraction of the analysis sample with acetonitrile/water, clean-up of ZEA with immuno-affinity column (IAK, R-Biopharm AG, Germany) that contains specific monoclonal antibodies, ZEA's elution from the column with acetonitrile and determination with reverse-phase liquid chromatography with flourescense detector (De Saeger *et al*. 2003). ZEA standards (Sigma, USA) was also used as control for standardization of HPLC. Maximum absorbance measurement that is observed around 274 nm in a spectrophotometer wavelength of prepared stock solution was made and concentration was calculated (Fazekas and Tar, 2001).

Standard solutions were prepared at 5 different concentrations (25, 250, 500, 1000 and 1250 ng/ml) to form a calibration chart. Each concentration solution was injected 3 times to HPLC. Calibration chart was formed at the end of the analysis with the calculation of the areas obtained after each injection from extracts obtained from infected and control grains.

2.4. Statistical Analysis

The data were analyzed using SAS V8 statistical software package (SAS 1999). Analysis of variance was performed using the PROC GLM command and to compare the levels of found significant variance components analysis, least significant difference (LSD) test was utilized. In order to determine the relationship between the properties examined, Pearson correlation coefficients were determined by PROC CORR command.

3. RESULTS

All wheat genotypes developed FHB sysmptoms when inoculated with *F. graminearum* but there was wide range in susceptibility. Symptoms of FHB ranged from dark-brown to bleached spikelets. Spikelets with any of these symptoms was recorded as diseased. First signs were developed in the first week of infection, then progressed upwards and downwards of infected heads by time. The progress of the disease showed variation in different genotypes.

Figure 1. FHB symptoms developed 21 days after inoculation on wheat head (circled head).



When inoculated with F. graminearum, disease severity and relative head weight differed amoung wheat genotypes, between experimental years and between genotype x experimental years. ZEA accumulation was statistically different at different genotypes, also difference observed between genotype x experimental years. We did not find a difference between two experimental years in means of ZEA accumulation.

There were significant differences in disease severity among the fifteen wheat varieties after inoculation with *F. graminearum.* These differences were observed in both experiments. Disease severity was ranged between 24.7- 78.8% amoung the genotypes. The aggressiveness of the fungus towards Edirne, BBVD7 and Kate1 genotypes was great as more than 70% of the spikelets were infected. The most tolerant genotype was Golia; The mean value obtained from two years experiments, only 24.7% of spikelets showed FHB symptoms (Tables 1 and 2). The mean value of disease severity was as low as 24.7% in Golia.

The relative head weights were also affected by the infection ranging 34.1 to 78.4. Severely infected heads weighted lower than others, again, relative head weight as high as 78,4 in the most tolerent genotype, Golia.

The genotypes of the host influence the ZEA concentrations closely. More ZEA accumulation was measued in more damaged kernels, therefore low disease severity means low ZEA contamination. ZEA accumulation in grains ranged from 1.8 to 106.7 µg/kg**.**

A correlation analysis was performed to find whether a correlation exists amoung disease severity, relative head weight and ZEA accumulation in grains. We found a positive correlation between disease severity and ZEA accumulation, but negative correlation between disease severity and relative head weight (Table 3).

Table 1. Mean for disease severity, relative head weight, and ZEA concentration of 15 wheat genotypes.

	Disease Severity			Relative head weight			Zearelanone µg/kg		
Variety	1. year	2. year	Mean	1. year	2. year	Mean	1. year	2. year	Mean
Edirne	82,4 a	75,2 a	78,8 a	38,2 f	30,0 f	34,1 h	104,3 a	109,0 a	106,7 a
BBVD7	78,9 ab	74,6 a	76,7 a	38,3 f	40,9 e	39,6 fg	68,3 c	68,7 b	68,5 b
Flamura	73,8 b	66,3	70,0 bc	41,3 f	32,5 f	36,9 gh	91,3 ab	73,7 b	82,5 b
KateA1	72,7 c	74,4 ab	73,6 ab	43,5 ef	45,8 de	44,6 d-f	23,0 d-g	27,7 c-e	25,3 d-f
Yunak	64,9 cd	52,6 f	58,8 ef	45,3 d-f	44,1 e	44,7 d-f	77,3 bc	82,3 b	79,8 b
Prostor	64,8 cd	58 ef	61,4 d-f	46,0 c-f	43,9 e	44,9 de	14,0 eg	85,7 ab	49,8 c
Aksel	63,0 d	62,1 c-e	62,5 de	43,1 f	45,4 de	44,3 ef	39,3 d	39,7 c	39,5 cd
Gelibolu	61,9 d	68,4 b-e	65,2 cd	51,8 c-e	51,7 cd	51,7 c	26,3 d-f	22,3 c-f	24,3 d-f
Tina	61,4 d	59,5 df	60,5 ef	45,8 c-f	53,8 c	49,8 cd	33,7 de	30,7 cd	32,2 de
Konya	61,0 d	52,0 f	56,5 f	46,4 c-f	44,2 e	45,3 de	11,0 fg	10,0 d-f	10,5 fg
Dropia	60,9 d	63,3 c-e	62,1 d-f	52,6 cd	42,8 e	47,7 c-e	24,7 d-f	19,7 c-f	22,2 ef
Tosunbey	43,3 e	41,4 g	42,3 g	54,2 c	39,6 e	46,9 c-e	21,3 d-g	5,7 ef	13,5 fg
Sagittar	38,3 e	38,1 gh	38,2 gh	66,0 b	64,7 b	65,4 b	20,7 d-g	13,3 d-f	17 eg
Studnic	37,6 e	32,8 h	35,2 h	66,4 b	63,8 b	65,1 b	14,0 eg	10,3 d-f	12,2 fg
Golia	25,7 f	23,7 i	24,7 i	77,3 a	79,5 a	78,4 a	1,3 g	2,3 f	1,8 g
Mean	59,4 A	56,1 B	57,8	50,4 A	48,2 B	49,3	38 A	40,1 A	39,1

Means in columns followed by the same letter are not significantly different (P<0,05)

4. DISCUSSION

It is known that variability in pathogenicity exists in *F. graminearum* populations (Derviş, 2001; Panthi, 2012). The reactions of various wheat genotypes against a single *F. graminearum* could also be different. The results we obtained from our field trial confirmed the previous report as we also found great variation in means of disease severity amoung the wheat genotypes.

It was reported that aggressiveness of an isolate could depent on the host genotypes. An isolate can be highly virulent in one genotype; it could not cause disease at the same rate in another wheat variety (Ittu *et al*. 2000). But it was also reported that there is no interaction between the virulence of the isolates and also there is no relationship between the isolate and the variety (Gilbert *et al*. 2001). There are reports suggesting that *F. graminearum*, there is large genetic variation within a given population, even in samples collected from a small area within a field (McMullen, et al., 1997; Miedaner et al., 2010).

Relative head weight was affected negatively by inoculation of *F. graminearum* into 15 wheat genotypes. As it was expected the relative head weights reduced in the genotypes with high disease severity. These results were also observed in previous reports (Snijders and Perkowski 1990; Tunalı *et al*. 2006).

Table 2. Significance of mean square values for disease severity, relative head weight and ZEA concentration for each source of variation

SV	SD	Disease Severity	RHW	ZEA
REP	2	5,67	7,40	29,88
YEAR	1	235,2**	113,344**	92,01
G	14	1519,2**	836,06**	5959,73**
GXY	14	35,99**	51,79**	623,61**
Error	58	7,60	6,38	56,00

** Statistically different at P<0,01

Many studies have reported a positive correlation between disease severity and mycotoxin accumulations in cereals (Malbrán 2012; Puri and Zhong, 2010). Although disease development and toxin accumulation are influenced by rainfall, relative humidity and temperature, the strains do not produce toxins fail to infect or colonise the heads (Desjardins et al.,,1993) In our present study we found that there was great variation in ZEA accumulations in different genotypes. We found positive interactions between disease severity and Zearalenone production. Accumulation of ZEA was higher in highly infected genotypes.

The results obtained from this study contrasts with the reseach made previously by our laboratuary using *F. culmorum*. Gencer (2012) found that there was no correlation between disaese severity and ZEA accumulation in grains infected by *F. culmorum*.

In this study we found that *F. graminearum* isolates differ in ZEA production that high ZEA producers seems more aggressive than low ZEA producers. These results suggests that instead of using one single isolate, breeders should use mixtures of isolates to screen for FHB resistance in wheat lines.

Table 3. Correlation coefficient and severity levels related to the features examined in the trial.

		ZEA	RHW
	Year 1	0,68**	-0,92**
Disease Severity	Year 2	0,57**	-0,74**
	Mean	0,62**	-0,81**
	Year 1	-0,63**	
RHW	Year 2	-0,63**	
	Mean	-0,63**	

** Statistically different at P<0,01

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