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The Relation Between Virulence of Some Egyptian Isolates of *Fusarium graminearum* and Deoxynevalenol Production in Some Maize Cultivars

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Abstract: Sixteen different isolates ***Fusarium graminearum*** were isolated from various local cultivars of maize collected from different locations in Egypt. The isolates of *F. **graminearum*** were screaned for their ability to produce deoxynivalenol (DON) using solid state fermentation on rice medium. Fourteen isolates were DON producer and two isolates were DON non producer. Concentration of DON was estimated by visual comparison with known amounts of DON standard. The mean DON concentrations ranged from 22.3-375.2 µg/g. DON producing and non producing isolates of *F. **graminearum*** were tested for their ability to cause Gibberella ear rot in two susceptible maize cultivars. Harvested maize ears were analyzed for disease severity, grain yield and DON concentration. All the tested isolates were pathogenic, more aggressive isolates produced higher DON concentration which indicate that DON can play a role as virulence factor. The high DON producing isolate of *F. **graminearum*** was inoculated to six different maiz cultivars to determine when DON was detectable after inoculation and to estimate DON concentrations in inoculated maize kernels. Deoxynivalenol (DON) was detected in the different maize cultivars 48 h post inoculation (PI) and its accumulation peaked at 120 h and decreased after 240 h(PI). DON concentration in inoculated maize cultivars at 120 h (PI) ranged from 2.75-5.55 (µg/g), and differences among the cultivars were significant. Understanding when DON are synthesized in the local cultivars will help how to prevent or delay the biosynthesis of this toxin.

Key words : *F. **graminearum***, maize, virulence, deoxynivalenol

Introduction

Maize (***Zea mays***) is considered one of the promising cereal crops in Egypt (FAO, 1993). Maize ears are naturally contaminated with different fungi including ***Fusarium*** spp., e.g *F. **oxysporum*** schlecht. Emend. Snyd. (Hans; *F. **solani*** Mart. (Appl. (Wollen.); *F. **moniliforme*** var. ***subglutinans*** Wolen (Reink) and *F. **graminearum*** schwabe (El-Maghraby ***et al.***, 1995; and Fadl Allah, 1998). ***Fusarium graminearum*** schwabe (teleomorph = ***Gibberella zeae***) (schwein. Petch) is pathogenic on cereal species, such as wheat (***Triticum aestivum** L.*), barley (***Hordeum vulgare** L.*) causing Fusarium head blight and infect maize causing ear and stalk rot. Gibberella ear and stalk rot of

maize is characterized by pinkish to reddish coloured mold on the kernel as well as on the cob and husk tissues. The pathogen can reduce yield and quality of grain (Perry ***et al.***, 1995). The reduction is partially a result of mycotoxins produced by the pathogen.

The pathogen produces trichothecene mycotoxins such as deoxynivalenol (DON) during parasitic and saprophytic metabolism in host tissues (Bennett ***et al.***, 1988; Mills, 1982; and Mirocha ***et al.***, 1994).

Trichothecenes belong to a family sesquiterpenoid secondary metabolites produced by ***Fusarium*** species (Vesonder and Golinski, 1989). These compounds are an important groups of mycotoxins that causes serious problems of food pollution, they have been implicated in incidents of mycotoxicosis such as vomiting, dermatitis and hemorrhagic septicemia in humans and livestock (Desjardins ***et al.***, 1993).

The phytotoxic nature of DON suggests that it may play a role in the pathogenicity. The role of trichothecenes has been investigated in a number of Fusarium diseases of crop plants (Desjardins and Hohn, 1997). The pathogenicity of ***F. graminearum*** was not prevented by the inability to produce trichothecenes, but the toxin may serve as a factor for aggressiveness or virulence of the pathogen (Desjardins ***et al.***, 1996). Because of the toxicological importance of DON, the objective of this study was to:

1) investigate DON production among some Egyptian ***F. graminearum*** isolates
2) Investigate whether isolates of ***F. graminearum*** differed in DON production have different level of agressivness during infection of susceptible maize cultivars
3) Determine when DON is synthesized by ***F. graminearum*** after maize infection, which may enable us how to prevent or delay the biosynthesis of toxin in order to control the disease.

Materials and Methods

Isolate collection

Sixteen isolates of ***Fusarium graminearum*** were isolated from naturally infected maize ears collected from different locations of Egypt, (Table 1). Isolation was made according to the procedure of (Wilcoxson ***et al.***, 1988). Identification of the isolates of ***F. graminearum*** (teleomorph= ***Gibberella Zeae***) followed the taxonomic system of (Nelson ***et al.***, 1983). The isolated strains were grown on PDA plates and incubated at 25°C in the dark for 10 days. For culture preservation the isolates grown on PDA were transferred aseptically in PDA slants and kept in the refrigerator until assayed.

In vitro DON Production

The isolated strains of ***F. graminearum*** were screened for their ability to produce DON using solid state fermentation on rice medium according to (Abbas ***et al.***, 1984). Rice medium was

prepared by weighing 100 g of polished rice into 500 ml Erylenmeyer flasks containing 125 ml of distilled water and left overnight. Flasks containing rice were autoclaved at 121°C for 20 min. Autoclaved rice was inoculated with 3 mm diameter agar plugs from each of the tested isolates of *F. graminearum* that were maintained on PDA. One flask of rice medium was utilized as a noninoculated control. Inoculated flasks were incubated at 25°C for 4 weeks in the dark. Flasks were shaken once every day to prevent rice adhering. At the end of the incubation period, cultures were removed and dried in a fume hood for 5 days. The dried rice culture were finely ground using an electric mill and the fine powder was kept at 4°C for DON analysis. Uninoculated autoclaved rice treated the same and served as control.

Deoxynivalenol was determined according to Truckess ***et al***, (1984). A sample of 25 g was extracted with acetonitrile - water (84 : 16 v/v) and 60 ml hexane. The mixture was blended for 2 min at high speed and filtered through filter paper (Whatman No. 1). twenty ml of the filtrate were cleaned on a chromatographic column with 0.1 g of celite as filtering base and a mixture of 1.5 g activated charcoal - neutral alumina - celite in g (0.7 : 0.5 : 0.3) was added to the tube.

After extract elution, 10 ml actonitrile - water (84 : 16 v/v) were added for rinsing the column collected in the same round bottom flask. This filtrate was evaporated to dryness on a rotatory evaporator. Residue was redissolved in 100 (l of chloroform : acetonitrile (4:1v/v) and DON was assayed by thin layer chromatography using TLC plates (0.25 mm, Merck). The development solvent was toluene - ethyl acetate - formic acid (5 : 4 : 1 v/v). DON was detected under long wave UV light after spraying the plates with 20% aluminum chloride in ethanol solution and heated at 100°C for 15 min to produce a blue flourescent spot. DON concentration was determined by visual comparison with known amounts of DON standard obtained from the Sigma Chemical Company (USA). For DON confirmation a second spray and heating was carried out (20% sulfuric acid in ethanol and 110°C, 15 min), turning the DON spots brown.

Pathogenicity of isolates of *F. graminearum* and determination of DON production

Three isolates of *F. graminearum* with different levels of DON production (Code # 8 highly producing, Code # 11 moderately producing and Code # 4 non producing) were chosen to infect two susceptible local maize cultivars. Giza-2 and Baladi. The maize cultivars were planted in plastic pots (16 × 16 cm diameter × height) containing autoclaved sandy loam soil. Each pot was planted with three seeds and thinned to one plant at the three leaf stage.

Macroconidia were produced by culturing each of the 3 isolates of *F. graminearum* on PDA in pteri plates. Isolates were incubated for 15 days at 25°C in the dark. Macroconidia were harvested by adding 10 ml of sterile distilled water to each plate. Macroconidia were dislodged by gently stirring the flooded cultures with a sterile glass rod. Inoculum concentrations were adjusted to 10^7 conidia ml^{-1}.

The maize plants were inoculated through the silk channel technique according to (Reid ***et***

al., 1996) the inoculum involved the injection of a 2 ml spore suspension into the silk channel (inside the husk cavity and above the cob) of the primary ear 7 days after silk emergence. This was accomplished using a disposable 10 ml syringe. Maize plants inoculated with water served as control. After 6 weeks of inoculation, ears were harvested. Disease intensity evaluation was based on a visual estimation of the percentage of visibly infected kernels on an ear as follows, 1 = 0%, 2 = 1-3%, 3= 4-10% 4= 11-25%, 5=26-50% 6=51-75% and 7=76-100%, (Reid ***et al.***, 1992). After drying harvested kernels of each treatment were weighed. 100 g samples of each treatment were dried at 50°C for 3 days, then ground to fine powder for later extraction and DON analyses following the procedure described earlier.

Determination of DON synthesis on inoculated different cultivars of maize over different time intervals

The experiment was conducted using 6 cultivars of maize namely (Baladi, Giza-2, Rg-15, Rg-42, S.c.10, and D.c 204) Maize cultivars were kindly supplied by Maize Research section, Agriculture Research Center Giza-Egypt. Six pots of each cultivar were planted as previously described and inoculated with the high DON producing isolate (Code # 8) and one additional pot was used as water - inoculated control. Each pot of each cultivar was considered as a replicate. Samples were taken at 24 h intervals post inoculation, from 0 -240 h .

Statistical analysis

The results obtained were subjected to statistical analysis using the Duncan`s multiple range test according to Snedecor and Cochran (1989).

Results and Discussion

The concentrations of deoxynivalenol (DON) produced by the tested isolates of *F. graminearum* grown on autoclaved rice as determined by visual comparison with known amount of DON standard are illustrated in Table 1, the results indicated that 14 of the 16 isolates of *F. graminearum* examined produced DON. whereas two isolates was not able to produce DON. Data presented in Table 1 showed that *F. graminearum* tested isolates produced DON at concentrations ranging from 22.3 - 375.2 µg g^{-1}.

The results indicate variation among *F. graminearum* isolates with respect to DON production. This result is in agreement with the work of Mirocha ***et al***, (1989), they found that 95% of 114 *F. graminearum* isolates collected from soil or cereal plants on a world wide basis were capable of producing DON ***in vitro***.

The three isolates of *F. graminearum* with different levels in DON production (Code # 8, high DON produces, Code # 11 moderate producer and Code # 4 non - producer, Table 1) were all able to infect the two susceptible maize cultivars (Giza 2 CV and Baladi) and caused visible ear rot symptoms (Table 2). Inoculation of both maize cultivars (Giza 2 CV and Baladi) with DON

producing isolates (Code # 8, high DON producer and Code # 11, moderate producer) resulted in higher proportion of severely diseased ears, disease rating 6.3 and 5.9 respectively for cultivar Giza 2 CV, and caused significantly more disease than the DON non producing Code # 4. However the mean disease rating for cultivar (Baladi) ears inoculated with Code # 8 and 11 were 6.1 and 4.4, respectively and 2.2 with Code # 4. (Table 2).

Table 1: Concentrations of deoxynivalenol (DON) produced by different isolates of ***Fusarium graminearum*** isolated from maize kernels collected from different Governorate, Egypt

Fusarium graminearum code	Locality	DON Concentration µg g^{-1}
Code1	Gharbia	22
Code 2	Menoufia	46
Code 3	Giza	195
Code 4	Gharbia	-ve
Code 5	Giza	131
Code 6	Giza	207
Code 7	Sohag	95
Code 8	El-Minia	375.2
Code 9	El-Minia	235
Code 10	Assuit	150.6
Code 11	Cairo	106.3
Code 12	Assuit	263.5
Code 13	Cairo	54.9
Code 14	Giza	-ve
Code 15	Menoufia	112
Code 16	Gharia	104

-v= not detected

Table 2: Mean values of disease rating, kernel yield and dexynivalenol (DON) content produced by three *Fusarium graminearum* isolates in inoculated kernels of Giza-2 and Baladi maize cultivars

	Maize cultivars					
	Giza-2			Baladi		
F. graminearum Isolates	Disease Rating	Yield Kernel weight ear^{-1} (g)	DON content µg g^{-1}	Disease Rating	Yield Kernel weight ear^{-1} (g)	DON content µg g^{-1}
Water inoculation control	1.17E	58.32B	0.01E	1.10E	60.88A	0.01E
Fig.8 highly DON producer	6.27A	24.67H	40.55A	6.15A	33.17G	38.63A
Fig.11 moderatly DON producer	5.87A	38.20F	32.42B	4.40B	40.47E	27.85C
Fig.4 Non- DON producer	3.50C	43.88D	1.75E	2.21D	52.78C	6.07
L.S.D at 0.05	0.310	1.060	1.756	0.4387	1.499	2.484

The means followed by the same letter are not significant

Table 3: Mean yield of deoxynivalenol (DON) in inoculated kernels of six cultivars of maize which were inoculated with Fig. 8 (DON high producer isolate) of ***F.graminearum*** after different time intervals

	Maize cultivars Time Intervals (h)				
DON µg/g	48 h	72 h	120 h	240 h	Cultivar Mean
Giza-2	1.32G	3.55D	5.55A	4.37B	3.70
Baladi	0.80HI	1.82F	3.52D	4.10BC	2.56
Rg-42	0.40I	1.52FG	3.35D	2.52E	1.95
Rg-15	0.70HI	2.52E	4.47B	3.37D	2.78
Sc-10	0.72HI	1.52FG	3.77CD	3.60D	2.40
Dc-240	0.42I	0.85H	2.75E	1.70FG	1.42

L.S.D at 0.05, Cultvar (A) 0.1906, Treatment (B) 0.1556, Cultivar × treatment (AXb) 0.3811, The means followed by the same letter are not significant

The high and moderate DON producing isolates Code # 8 and 11, respectively significant decrease yield relative to DON non producing Code # 4 (Table 2). Compared with cultivar (Baladi), cultivar (Giza 2) showed greater differences in yield between DON producing isolates and DON non producing isolate.

High concentrations of DON (40.5 and 32.4 µg/g) were detected in maize cultivar Giza 2 inoculated with Code # 8 and Code # 11 respectively (Table 2). In case of cultivar (Baladi) DON concentration were (38.6 and 27.8 µg g^{-1}) produced by the same isolates. Kernels from cultivars (Giza 2) inoculated with the DON - non producing isolate (Code # 4) contained DON levels slightly above the water inoculated control level.

The present data demonstrated that the three isolates of ***F. graminearum*** with different levels of DON production were all pathogenic to susceptible maize cultivars. The observed differences in aggressiveness of isolates can be attributed to the different DON production rates. The decreased virulence of the isolate unable to produce DON implies that DON may support the infection of maize, while the quantity of DON produced seems to be unimportant. This finding agrees with the results of Proctor ***et al.*** (1995), Desjardins ***et al.*** (1996) and Harris ***et al.*** (1999).

These results indicated a relationship between the disease severity rating and DON concentration. The present results are an agreement with the work of Reid and Sinha (1998). They determined DON concentration in 10 maize hybrids using CD-ELISA method and found that hybrid lines exhibited similar disease severity rating accumulated similar concentrations of DON.

There were significant differences among the time intervals and DON accumulation in the 6 tested cultivars (Table 3). DON was detected in the tested cultivars at 48 h post inoculation (PI)

and gradually increase until 120 h, then decreased after 240 h.

The present data suggests DON are produced in our Egyptian maize cultivars much earlier than indicated previously by Miller ***et al.*** (1983). They reported that trichothecanes were synthesized in corn 14 days after inoculation, while in wheat it was synthesized 8 days from inoculation (Miller and Young, 1985). These results confirmed the previously report of Mirocha ***et al.*** (1997), they stated that using single spikelet analysis, DON is detectable as soon as 48 h after inoculation and the amount of DON in this very early phase of pathogenesis may contribute with aggressiveness of the isolate.

The primary objective of the time intervals experiment was to determine when DON could be detected in the different local cultivars in order to control the disease through the application of fungicide or biocontrol organisms at the proper time which interfere with the biosynthesis of DON.

It is concluded that, regarding the high percentage of DON producers among the 16 tested ***F. graminearum*** isolates, most natural ear rot epidemics should result in mycotoxin accumulation in grain. Therefore, the study of inheritance of resistance to ear rot among maize cultivars is needed to know the genetic mechanisms affect resistance to the disease and toxin accumulation.

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