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Head blight and biosynthesis of Fusarium toxins in barley kernels field inoculated with *Fusarium culmorum*

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Abstract

Heads of 12 spring barley genotypes (eight cultivars, four lines) were inoculated with conidial suspensions of two *Fusarium culmorum* isolates (I_1 and I_2). Both isolates caused the following significant reductions (%) when compared with the control: number of kernels head^{-1} 12 and 16, weight of 1000 kernels 47 and 24, yield 49 and 37, for $I_1$and I_2 respectively. Both isolates were able to produce average levels (mg kg^{-1}) of deoxynivalenol of 67.1 and 13.9, 3-acetyldeoxynivalenol of 9.4 and 1.4 and zearalenone of 0.3 and 0.4 for I_1 and I_2 respectively. In kernels of three genotypes inoculated with the same isolates, 15-acetyldeoxynivalenol at an average level (mg kg^{-1}) of 1.3 and 1.5 was detected, while in kernels of six genotypes inoculated with I_2, 0.2 of nivalenol was found. A significant correlation for yield and toxin level was found for less pathogenic *F. culmorum* isolate I_2.

Introduction

Fusarium culmorum (W.G.Sm.) Sacc., *F. graminearum* Schwabe and *F. avenaceum* (Fr.) Sacc., are the most common fungi responsible for Fusarium head blight in barley in Poland (Kiecana, 1986). This is in agreement with results of other investigations performed in Europe (Mesterhazy, 1974; Snijders, 1990), North America (Stack and McMullen, 1985), South America (Sisterna, 1980) and South Africa (Sydenham et al., 1989). Different *Fusarium* spp. are more or less common depending on environmental conditions; hot weather with limited precipitation favours *F. culmorum* head infection (Leiteritz and Focke, 1977). Abundant growth of *F. culmorum* can cause biosynthesis of deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (3-AcDON and 15-AcDON) and zearalenone (ZEA) (Scott, 1989; Gareis et al., 1989). Nivalenol (NIV) was also found in barley samples infected by *F. culmorum* (Perkowski et al., 1992; Mirocha et al., 1994). Trichothecenes not only contaminate cereal grain but are an important factor in pathogenesis. Resistance of different cereal genotypes (Schroeder and Christensen, 1963; Miller et al., 1985) as well as differences in the pathogenicity of fungi of the same species play important roles in pathogenesis (Snijders and Perkowski, 1990; Mesterhazy and Bartok, 1993). The aim of this study was to compare resistance in 12 barley genotypes after inoculation with two *F. culmorum* isolates differing in pathogenicity and to determine possible correlations between yield, toxin formation and the isolate used.

Materials and methods

Source of barley kernels

Eight cultivars (Apex, Aramir, Ars, Bielik, Diva, Havila, Koru, Roland) and four lines (MOB-487, NAD-685, NAD-785, POB-984) of spring barley were inoculated under field conditions in experimental station RZD Czesławice near Lublin, Poland. Barley cultivars used in the inoculation experiment are very common in the region of Lublin (South – East part

of Poland) and originally were introduced in: Poland (cultivars Bielik and Ars), The Netherlands (Apex, Aramir, Diva and Havila), Sweden (Roland) and UK (Koru). All inoculated lines were of Polish origin and were tested in The Cereal Breeding Station, Czesławice, Poland. Two isolates of *F. culmorum* N° 158 = I_1 and 21 = I_2 were used for inoculation. The isolates were obtained from the culture collection at the Department of Plant Pathology, Agricultural University of Lublin, Poland. Pathogenicity of the above isolates was tested by the germination potential of Apex cultivar kernels, after inoculation with *F. culmorum* isolates. Isolates #158 and #21 reduced seed germination ability up to 4 and 5% respectively of germinating seedlings. Inoculum was prepared according to the method of Mesterhazy (1978) with our own modification. Growing medium (1 L) was prepared of water, starch (2 g), water extract of 0.5 kg barley leaves, and was autoclaved (1 h, 121 °C and 1 atm) before use. *F. culmorum* isolates were grown on the above medium according to the method described earlier (Kiecana, 1988). Cooled medium was inoculated with mycelium of two-week old cultures of isolates #158 and #21, and was incubated for two weeks at a temperature of 18–20 °C with natural (ambient) light. After incubation, stirred (10 min) inoculum was filtered through cheese-cloth, and the supernatant of the conidial suspension (5×10^6 spores ml^{-1}) was used for inoculation.

Each barley cultivar was sown April 19, 1991 and grown separately on 10 m^2 experimental plots bordered with rows and paths. Eighty heads of barley (20 heads per replicate) were inoculated with *F. culmorum* 4 days after anthesis (stage 10.5 on the Feekes scale) of 50 plants (July 1–5, 1991). Inoculum prepared as described above (1 ml $head^{-1}$) was applied with a laboratory sprayer. The same cultivars, grown the same way, but sprayed with distilled water instead of inoculum, were used as a control group. After inoculation, or water spraying, to avoid water evaporation and spread of inoculum, the heads were protected with plastic bags for 24 h. Weather conditions during the experiment are presented in Table 1. Mature heads were collected on August 19, 1991 and threshed manually. Yield (Y), number of kernels 10 $heads^{-1}$ (NK) and 1000 kernel weight (TKW) were measured for four replicates separately and were statistically correlated with results for content of Fusarium toxins in kernels (4 replicates).

Chemical analysis

Fusarium toxins (DON, 3-AcDON, 15-AcDON, NIV and ZEA) were extracted from finely ground samples as described by Tanaka et al. (1985). Trimethylsilyl (TMS) esters of the metabolites were prepared as described by Scott et al. (1986) and were analyzed by gas chromatography with ^{63}Ni electron capture detection (GC-ECD) after splitless injection on a Shimadzu C-R4Ax Chromatopac Gas Chromatograph fitted with a 25 m × 0.25 mm Permabond SE-54-DF-0.25 column (Machery Nagel). The carrier gas was helium (80 kPa) and the make-up gas nitrogen (45 ml min^{-1}). Injection temperature was 250 °C, while the temperature programme for the column was 60 °C for 1 min., then raised to 120 °C (10 °C min^{-1}), then raised to 280 °C (4 °C min^{-1}) and held at this temperature for 20 min. Detector temperature was 330 °C. Retention times (min.) were as follows: DON – 29.60; 3-AcDON – 35.75; 15-AcDON – 37.05 and NIV – 40.90. Confirmation of the presence of metabolites and their quantification was performed by capillary gas chromatography combined with a selective mass detector (ion trap) working in CI – mode with methanol as a reagent gas. Trifluoroacylated derivatives were prepared in reaction with trifluoroacetic acid anhydride as described by Schwadorf and Müller (1991). Detection limit for the metabolites was 1–5 $\mu g\ kg^{-1}$, depending on toxin analyzed and content of impurities in extracts. Recoveries (from barley spiked with toxic metabolites) were 78–89%. Part of the Florisil column eluate (Tanaka et al., 1985) was subjected to ZEA analysis by fluorescence detection after high-performance liquid chromatography (HPLC) on a Lichrosorb Si 60 column (5μm, 25 cm × 4 mm, Merck) at 25 °C with mobile phase (water – saturated dichloromethane/1-propanol, 98.5 : 1.5 v/v), flow rate 2 ml min^{-1}. A fluorescence detector (Model 1046 A, Hewlett Packard, Germany) at an excitation 236 nm and emission 450 nm wavelength was used. 'External standard' method with 'valley-to-valley' integration was selected. Retention time of ZEA was 4.6 min. while the detection limit was 0.5 $\mu g\ kg^{-1}$.

Results

Environmental conditions

Rainfall, during the field experiment, was below average for this region except during August when average humidity was 3% higher than the mean (Table 1).

Table 1. Air temperature, rainfall and relative air humidity recorded in 1991 at Czesławice Cereal Breeding Station

Month	Long term (1963–1990) average data		Air temperature[a] (°C)	Percentage of average value of rainfall	Range of relative air humidity (%)
	Air temp. (°C)	Rainfall (mm)			
April	7.5	43.3	−0.4	36	54–92
May	13.4	61.6	−2.8	91	62–91
June	16.4	79.6	−0.8	103	66–88
July	17.8	80.8	+0.9	59	60–91
August	17.1	72.2	+0.6	58	66–92

[a] Mean deviation of average.

Agronomic performance

All barley genotypes inoculated with either *F. culmorum* isolate exhibited a significant number of bleached heads, which are typical symptoms of fusariosis. Kernels collected from infected heads were small, light and shrivelled, while kernels from the control group did not exhibit any symptoms. Yield (Y), number of kernels head^{-1} (NK) and weight of 1000 kernels (TKW) were used as criteria of genotype resistance to fusariosis. All results indicated that the two isolates of *F. culmorum* used for inoculation were different (Table 2). Reduction (%) of Y (49.3) and TKW (47.2) for I_1 isolate were higher when compared with I_2, respectively 36.7 and 24.3%. Number of kernels head^{-1} was reduced at a similar rate for I_1 (12.3%) and I_2 (16.3%) isolates.

All yield indices (Y, NK, TKW) for I_2 isolate, and TKW and Y were significantly correlated only after inoculation with I_1 isolate (Table 3).

Chemical analysis of the mycotoxins

The following toxic secondary metabolites: DON, 3-AcDON, 15-AcDON, NIV and ZEA were detected in kernels of inoculated barley (Table 4). Samples inoculated with I_1 isolate were particularly highly contaminated. The average concentration of DON was 67.1 mg kg^{-1} while of 3-AcDON was 9.4 mg kg^{-1}. In all samples ZEA was present (mean 0.32 mg kg^{-1}) but only three samples were contaminated with 15-AcDON (average 1.3 mg kg^{-1}). After inoculation with I_2, except for ZEA (0.35 mg kg^{-1}), the average concentrations (mg kg^{-1}) of toxic metabolites were much lower: DON – 13.9; 3-AcDON – 1.4; NIV – 0.16. Levels of kernel contamination with toxic metabolites were correlated for I_1 and I_2 isolates and the coefficients are presented in Table 5. As in case of yield indices, this correlation was significant for isolate I_2.

Table 2. Average reduction (%) of yield, 1000 kernel weight and number of kernels head^{-1} of 12 barley cultivars and lines inoculated with two *F. culmorum* isolates (F.c. nr 158 = I_1 and nr 21 = I_2)

Cultivar or line	Reduction (%)					
	Yield (Y)		1000 kernel weight (TKW)		Numbers of kernels head^{-1} (NK)	
	I_1	I_2	I_1	I_2	I_1	I_2
Apex	66.2	79.5	66.6	55.5	4.1	47.1
Aramir	48.3	26.7	57.5	31.2	1.5	1.2
Ars	55.7	52.0	64.0	21.0	2.6	28.6
Bielik	58.0	28.0	50.0	11.9	14.2	11.1
Diva	67.8	17.1	66.6	7.0	12.1	1.8
Havila	49.0	45.6	36.2	32.3	12.4	21.7
Koru	32.2	46.0	26.8	42.0	14.0	16.1
Roland	46.0	21.4	33.0	13.6	17.1	7.3
MOB-487	55.7	39.1	48.5	21.5	23.1	16.7
NAD-685	50.9	24.6	53.6	21.1	15.6	7.7
NAD-785	24.2	34.4	28.6	23.4	12.5	9.2
POB-984	31.8	31.8	16.4	21.3	16.2	26.6
Mean	49.3	36.7	47.2	24.3	12.3	16.3

Table 3. Correlation coefficients between yield (Y), 1000 kernel weight (TKW) and number of kernels head^{-1} (NK) for 12 barley cultivars and lines inoculated with two *Fusarium culmorum* isolates (F.c. nr 158 = I_1 and F.c. nr 21 = I_2)

	Correlation coefficient $I_1 \neq I_2$			
	I_1		I_2	
	Y	TKW	Y	TKW
TKW	0.8591[a]	–	0.9061[a]	–
NK	0.2436	−0.1285	0.9482[a]	0.8268[a]

[a] significant at $p < 0.01$.

Discussion

To our knowledge, the present paper is one of the first reports on agronomic performance, genotype resis-

Table 4. Concentration (mg kg^{-1}) of deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NI and zearalenone (ZEA) in 12 barley genotypes (8 cultivars and 4 lines) inoculated with two *Fusarium culmorum* isolates (F.C. no 158 = I_1 and F.c. no = I_2)

Cultivar or line	Concentration (mg kg^{-1}) of Fusarium mycotoxin									
	Isolate									
	I_1					I_2				
	DON	NIV	3-AcDON	15-AcDON	ZEA	DON	NIV	3-AcDON	15-AcDON	ZEA
Apex	33.00	ND	6.86	ND	0.29	15.80	ND	2.03	ND	0.54
Aramir	150.37	ND	16.67	0.71	0.45	5.15	0.09	0.36	ND	0.17
Ars	136.77	ND	19.86	0.63	0.14	9.97	0.04	1.04	ND	0.13
Bielik	133.07	ND	28.89	ND	0.17	9.95	0.04	1.49	ND	0.18
Diva	50.10	ND	9.62	2.55	0.15	54.05	0.63	4.84	1.46	0.81
Havila	3.04	ND	3.21	ND	0.15	9.50	ND	1.27	ND	0.27
Koru	11.93	ND	2.16	ND	0.54	1.91	ND	0.16	ND	0.14
Roland	1.39	ND	ND	ND	0.18	7.72	ND	0.47	ND	0.44
MOB-487	177.75	ND	5.77	ND	0.86	8.84	ND	1.68	ND	0.28
NAD-685	37.46	ND	4.99	ND	0.45	12.07	0.10	0.89	ND	0.36
NAD-785	64.30	ND	4.60	ND	0.36	19.18	0.03	1.45	ND	0.66
POB-984	6.01	ND	0.56	ND	0.10	12.00	ND	1.00	ND	0.19
Mean	67.10	ND	9.38	1.30	0.32	13.85	0.16	1.39	1.46	0.35

ND – not detected.

Table 5. Correlation coefficients between concentrations of deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON) and zearalenone (ZEA) for 12 barley cultivars and lines inoculated with two isolates of *F. culmorum* (F.c. nr 158 = I_1 and F.c. nr 21 = I_2)

Correlation coefficient $I_1 \neq I_2$ ($p < 0.05$)				
	I_1		I_2	
	DON	3-AcDON	DON	3-AcDON
3-AcDON	0.7025[b]	–	0.9495[a]	–
ZEA	0.4374	−0.1717	0.8084[a]	0.7359[a]

[a] significant at $p < 0.01$.
[b] significant at $p < 0.05$.

tance, and formation of toxic secondary metabolites in barley inoculated with *F. culmorum*. Kernels of the 12 barley genotypes and lines tested exhibited typical symptoms, described above, of fusariosis. The kernels were damaged, small and shrivelled, resulting in poor yield indices. Similar observations have been reported for artificially inoculated wheat (Snijders and Perkowski, 1990) and barley (Mirocha et al., 1994); in the latter publication a 20% incidence of necrotic spikelets, in four barley cultivars inoculated with *F. culmorum*, was reported.

Average reduction (%) of all yield indices, calculated for both isolates I_1 and I_2, was as follows: Y – 43; TKW – 36; NK – 14 and was comparable to those reported previously for wheat and triticale (Mesterhazy, 1978, Kiecana et al., 1988). Reduction of yield was the lowest in cv. Roland and in lines NAD – 785 and POB – 984, and the highest in cvs. Apex and Ars. Reduction of Y and TKW was higher after inoculation with I_1 isolate, which confirms an earlier reported relationship of fusariosis severity and aggressiveness of pathogen (Snijders and Perkowski, 1990). After inoculation of barley genotypes/lines with a moderately pathogenic strain I_2, a significant correlation ($p < 0.01$) between all yield indices was observed (Table 3). No correlation was observed for yield indexes (Y, NK, TKW) of barley inoculated with both isolates I_1 and I_2 because of different genotype/line resistance to isolates of different pathogenicity (Schroeder and Christensen, 1963, Miller et al., 1985, Snijders and Perkowski, 1990). Kernels of all inoculated barley cultivars and lines were contaminated with DON. The highest level of the metabolite concentration (177.8 mg kg^{-1}) was found in kernels of line MOB – 487 inoculated with I_1 isolate, while an average concentration, for all cultivars/lines inoculated with the same strain, was 67.1 mg kg^{-1}. Such high levels of DON, after barley or wheat inoculation with *F. culmorum* were reported previously by Mirocha et al. (1994) and by Snijders and Krechting (1992). The trichothecene 3-AcDON was analyzed at a high

average concentration of 9.4 mg kg^{-1}. Three of the inoculated samples with I_1 contained both acetylated derivatives of vomitoxin, 3-AcDON and 15-AcDON. Previously the presence of each derivative was related to geographic location; 3-AcDON was formed by *F. culmorum* strains of Europe and Far East origin while the latter metabolite was formed by fungal isolates of North American origin (Yoshizawa and Morooka, 1975). Recently, co-occurrence of 3-AcDON and 15-AcDON has been reported in wheat, corn and barley (Perkowski et al., 1990a; 1991; 1992). Average contamination levels of samples inoculated with I_2 (DON 13.9; 3-AcDON 1.4 mg kg^{-1}) was 5–7-fold lower when compared with samples inoculated with I_1, and are similar to earlier findings in samples of wheat, rye and triticale after inoculation with moderately pathogenic *F. culmorum* isolates (Kiecana et al., 1988; Snijders and Perkowski, 1990; Perkowski et al., 1995). The amount of DON formed in barley genotypes inoculated with strain I_2 was limited and/or related to the level of NIV. Recent studies revealed that some isolates of *F. culmorum* are able to produce NIV as well as DON (Perkowski et al., 1992; Mirocha et al., 1994). Levels of kernel contamination with toxic metabolites of plants inoculated with I_1 and I_2, were correlated, and the coefficients are presented in Table 5. As for the yield indices (Y, NK, TKW), this correlation was significant for the I_2 isolate. In case of the strongly pathogenic I_1 isolate, only concentrations of DON/3-AcDON were significantly correlated ($p < 0.05$). The results also confirm the relationship presented above between fusariosis severity and aggressiveness of the pathogen as do earlier findings (Perkowski et al., 1990b, 1995; Perkowski and Chełkowski, 1993). Differing pathogenicities of *F. culmorum* isolates reported in previous papers (Snijders and Perkowski, 1990; Mesterhazy and Bartok, 1993) was also confirmed in this investigation. Agronomic performance in field experiments and the accumulation of toxic metabolites indicate that of the 12 barley cultivars and lines inoculated with *F. culmorum*, the most resistant to fusariosis were Roland, POB-984, Koru and Havila, while Ars, MOB-487, Diva, Apex and Bielik were the most susceptible to *F. culmorum* development, yield losses and biosynthesis of toxic metabolites by the fungus.

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