Screening of winter wheat breeding lines for resistance to Fusarium head blight and accumulation of *Fusarium* metabolites in grain

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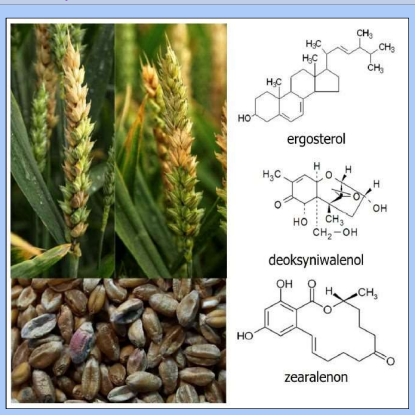
Introduction

Fusarium head blight is a disease of cereals caused by complex of fungi of the genus *Fusarium* (mainly *F. culmorum* and *F. graminearum*). These fungi produce toxic metabolites - mycotoxins. Spike infection by Fusarium leads to kernel damage and accumulation of mycotoxins in grain. In the case of consumption of food or feed produced from infected grain mycotoxins pose a threat to human and animal health. Due to the quantities determined in the cereal grain the most important mycotoxins are deoxynivalenol (DON) and zearalenone (ZEA). The resistance of wheat to FHB consists of several types (mechanisms) of resistance: resistance to infection (type I), resistance to disease spread (type II), kernel damage resistance (type III), tolerance on the accumulated toxins (low kernel damage or low yield reduction despite a severe spike infection or high content of toxins in grain (type IV) and resistance to accumulation of toxins in grain (type V) through their chemical modification (class 1) or blocking the synthesis (class 2) (Mesterhazy 2002, Boutigny et al. 2007, Foroud and Eudes, 2009).



Material and methods

In the field experiments in Radzików and Cerekwica the resistance to FHB of 224 lines and 10 check varieties/lines of winter wheat was assessed. These lines originated from wheat breeding programs (153 lines - DW 2014) and from the collection established by studies on the resistance to FHB in the years 2008-2013 (72 lines – 'resistant'). The control cultivars were: high yielding cvs 'KWS Ozon', 'Patars', 'Tonacja'; lines/cultivars highly resistant to FHB - 'A40-19-1-2', 'UNG 136.6.1.1', 'Arina', '20828'; lines highly susceptible to FHB in 2013 - 'SMH 8694', 'SMH 8816', 'NAD 10079'. Wheat spikes were sprayed at flowering with *F. culmorum* spores. The disease was first rated at about 10 days after the last inoculation. Fusarium head blight (FHB) was scored based on the mean percentage of blighted spikelets per infected spike (disease severity) and the percentage of infected spikes per plot (disease incidence). Fusarium head blight index was calculated as the combination of disease severity and disease incidence. After the harvest, the proportions of the *Fusarium* damaged kernels (FDK) was evaluated (resistance type III).

For 61 selected genotypes the contents of ergosterol (ERG) (resistance of type III) and trichothecenes B (deoxynivalenol [DON], 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and nivalenol) in the grain (type IV resistance) was analysed using the HPLC and GC techniques. The content of zearalenone (ZEA) was determined using enzyme-linked immunosorbent assay AgraQuant® ZON (type IV resistance).

For 42 genotypes resistance of type I and type II was additionally evaluated using spray and point inoculations.

No.	Line/cultivar	Type I resist. (#IP)	Type II resist. (#IS)	Type I+II combined	FHBi (%)	FDK (%)	ERG (mg/kg)	DON (mg/kg)	ZEA (µg/kg)	ERG/ DON
1	A 40-19-1-2 (R)	1,0	1,1	1,1	6,6	20,8	8,1	4,055	197	2,01
2	UNG 136.6.1.1 (VR)	2,0	1,1	1,6	6,8	13,1	13,9	7,888	196	1,76
3	STH 9059	2,3	1,6	2,0	9,6	19,5	15,9	8,189	193	1,94
4	NAD 11017	-	-	-	13,0	31,8	15,1	10,090	103	1,50
5	20828 (R)	1,0	1,7	1,4	9,1	23,7	14,6	8,510	351	1,71
6	KBP 1040	1,3	2,0	1,7	13,5	28,0	20,4	8,912	331	2,29
7	LAD 463/05	1,0	2,6	1,8	12,4	23,7	17,0	9,759	289	1,74
8	MOB 5578/06	1,0	1,9	1,5	12,8	37,4	20,0	10,710	360	1,87
9	C 3779/10	-	-	-	13,6	23,2	20,3	12,540	207	1,62
10	POB 0212	1,3	3,0	2,2	17,7	45,7	26,6	11,212	377	2,37
11	KBP 04.164	2,7	2,0	2,3	9,3	43,2	18,8	12,993	262	1,45
12	STH 1144	-	-	-	10,3	33,1	18,5	14,894	48	1,24
13	C 1/10	-	-	-	22,7	48,6	22,4	15,252	134	1,47
14	MOB ZB 301206	2,3	1,1	1,7	13,3	28,5	22,4	13,455	387	1,67
15	Arina (R)	2,0	1,7	1,9	8,3	32,3	17,2	15,859	126	1,09
16	KBP 15.221	1,7	2,1	1,9	12,8	31,2	22,5	14,523	359	1,55
17	KBP 1058	1,3	1,2	1,3	8,2	24,7	16,4	15,267	281	1,08
18	STH 008	1,3	3,3	2,3	10,6	27,2	19,6	16,775	186	1,17
19	KBP 1013	-	-	-	13,9	44,0	27,5	17,977	186	1,53
20	DED 389/-6	1,7	1,9	1,8	11,3	36,6	26,9	15,994	487	1,68
21	KBP 0820	3,0	2,2	2,6	12,8	36,6	29,1	17,062	370	1,71
22	POB 262/07	3,7	2,0	2,8	16,1	34,7	32,1	15,851	632	2,03
23	STH 9007	2,0	1,3	1,7	15,5	40,4	27,2	13,418	955	2,03
24	KOH 275	-	-	-	15,2	31,2	19,0	16,898	586	1,12
25	STH 105	1,3	3,3	2,3	16,9	45,7	30,1	19,243	319	1,57
26	POB 0713	-	-	-	20,5	49,6	37,7	19,637	300	1,92
27	SMH 7983	1,0	2,1	1,6	11,8	43,7	28,1	14,985	970	1,88
28	DM 3873/10	3,0	2,0	2,5	14,5	53,1	25,0	17,834	687	1,40
29	STH 032	2,7	3,1	2,9	15,9	38,2	35,6	15,232	1030	2,34
30	POB 0111	1,0	2,0	1,5	10,6	39,4	26,9	21,814	267	1,23
31	DM 2566/11	-	-	-	13,8	33,4	25,5	17,562	853	1,45
32	POB 3013/10	-	-	-	15,7	36,0	26,1	15,591	1168	1,68
33	STH 188	-	-	-	16,7	50,5	36,5	20,322	726	1,80
34	POB 457/07	2,0	1,4	1,7	15,0	39,7	33,2	20,783	684	1,60
35	C 41/10	-	-	-	18,2	48,1	28,2	24,399	242	1,15
36	STH 102 (Tox)	1,7	3,2	2,4	17,3	50,3	38,2	18,314	1065	2,09
37	DD 137/10-4	-	-	-	14,2	49,3	30,3	20,206	908	1,50
38	DC 332/09-3	2,7	2,3	2,5	14,2	48,9	34,5	22,664	597	1,52
39	C 4573/10	-	-	-	17,3	50,7	36,2	26,876	93	1,35
40	SMH 7974	1,7	2,3	2,0	13,4	43,1	25,6	18,916	1213	1,35
41	AND 143/10	-	-	-	13,5	44,4	30,0	24,507	514	1,23
42	POB 0112	2,3	2,5	2,4	17,1	50,8	22,4	15,297	1727	1,47
43	KBP 1017	-	-	-	21,0	55,7	31,1	23,898	812	1,30
44	KBP 1034	4,0	1,4	2,7	20,8	55,1	30,5	27,092	488	1,12
45	DCh 4763/07	1,7	1,6	1,6	11,0	44,1	33,9	22,773	1120	1,49
46	POB 0211	1,0	1,6	1,3	13,4	40,2	33,2	27,686	617	1,20
47	KWS Ozon (wz)	-	-	-	30,4	58,9	58,0	25,315	1020	2,29
48	Tonacja (wz)	1,7	2,9	2,3	20,4	55,4	36,3	28,168	672	1,29
49	STH 087	1,7	2,6	2,1	16,8	42,1	38,1	21,104	1568	1,80
50	Patras (wz)	-	-	-	25,1	50,9	30,0	26,094	1011	1,15
51	SMH 8553	1,0	1,9	1,5	15,2	47,8	29,0	26,663	1084	1,09
52	DM 3131/10 (Tox)	1,7	2,8	2,2	14,1	57,6	51,6	32,520	856	1,59
53	DM 2728/09 (Tox)	1,0	2,5	1,8	18,1	54,1	47,9	28,156	1466	1,70
54	KBP 0936	-	-	-	16,0	40,6	35,3	26,448	1852	1,33
55	AND 1055/02	1,0	2,3	1,7	9,9	37,8	32,4	31,812	1362	1,02
56	POB 0911	1,3	2,5	1,9	16,6	62,9	59,0	36,043	1140	1,64
57	SMH 8816 (S)	3,0	3,1	3,1	27,3	70,1	79,5	45,779	1845	1,74
58	DD 548/09 (Tox)	2,0	1,6	1,8	19,3	52,3	55,5	26,525	4522	2,09
59	NAD 11100	-	-	-	21,5	41,5	34,7	58,654	1814	0,59
60	NAD 10079 (S)	3,3	3,3	3,3	34,5	62,3	59,9	34,474	6450	1,74
61	SMH 8694 (S)	2,7	2,2	2,4	29,7	64,3	67,8	39,956	6190	1,70
	Means	**1,9**	**2,2**	**2,0**	**15,7**	**42,1**	**30,9**	**20,515**	**932**	**1,51**

Table 1. Resistance to Fusarium head blight (type I and II, FHBi, FDK) and accumulation of ergosterol (ERG) and *Fusarium* toxins (DON, ZEA) in grain of 61 lines and cultivars of winter wheat inoculated with *F. culmorum* in experiments in Radzików and Cerekwica. #IP – number of infection points, #IS - number of infected spikelets, FHBi – Fusarium head blight index, FDK – proportion of *Fusarium* damaged kernels; VR, R – resistant checks, S –susceptible checks, Tox – high toxin accumulation checks, wz – high yielding checks.

Results

The average severity of FHB was in Cerekwica FHBi = 23.9%, while in Radzików FHBi = 23.5%. There was no statistically significant difference between the FHB indexes in both locations. The correlation coefficient between FHB indexes in Cerekwica and in Radzików was significant (r = 0.605). The range of the reaction was in Cerekwica: 5.9% ('STH 1144') -72.5% ('KBP_09_38'); in Radzików: 0.5% ('UNG 136.6.1.1') – 54.7% ('KBP 11 21'). Mean FHBi for lines of DW2104 group was: 28.2% in Cerekwica and 26.8% in Radzików, while for genotypes from the group 'resistant' was: 15.1% and 16.4%, respectively.

The plant height in Cerekwica was significantly lower than in Radzików and was up to 75.0 cm (52.0-102.0 cm) and 105.4 cm (81.7-136.3 cm), respectively. Only three lines in Cerekwica were above 100 cm, while in Radzików it was 2/3 of the lines. Lines in the 'resistant' group had greater average plant height (94.2 cm) as compared to the DW2014 lines (88.5 cm). In both locations the relationships between the groups were the same. Plant height significantly, but weakly correlated with FHBi in Cerekwica (r = -0.240), while Radzików the correlation coefficient was highly significant (r = -0.628). The average plant height had a significant negative impact on the severity of the FHB (r = - 0.528) (Fig. 1).

Lines in the group 'resistant' were significantly less infected with FHB. In the DW2014 group, however, moderately resistant lines were also found, e.g.: 'STH 1144', 'POB 0513', 'STH 2041', 'NAD 11017'. Most of these lines, however, were characterized by a plant height above 90 cm. Dwarf lines (below 80 cm) had FHB indexes mostly above 25%. An exception were the lines 'DD 137/10', 'NAD 10041', 'STH 1124', 'DL 414/10', 'DL 414/10/6/3' of the Group DW2014 and 'CHD 6651/06' of the 'resistant' group

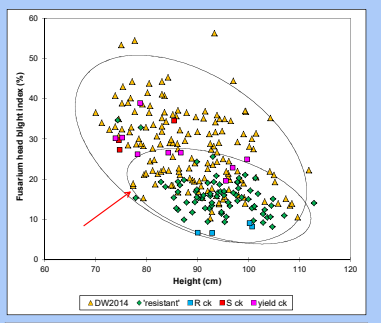


Figure 1. Relationship between plant height and Fusarium head blight index for 72 moderately FHB resistant lines ('resistant') and 152 novel breeding lines (DW2014) of winter wheat. Resistant lines shorter than 80 cm were pointed with arrow. R ck – resistant checks, S ck – susceptible checks, yield ck – high-yielding checks.

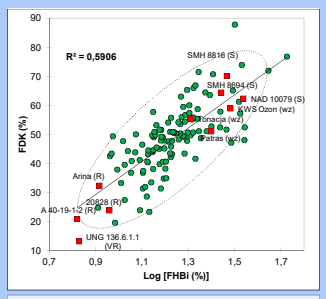


Figure 2. Relationship between log-transformed Fusarium head blight index (FHBi) and proportion of *Fusarium* damaged kernels for 126 lines of winter wheat. Mean values from experiments in Radzików and Cerekwica

The average FDK of 126 wheat lines on Cerekwica was 66.7%. The range of the reaction was from 19.1% ('UNG 136.6.1.1', 'C 3779/10') to 97.0% ('SMH 8644'). In Radzików the value of the FDK amounted to 30.0%. The range of the reaction was from 7.1% ('UNG 136.6.1.1', 'C 3779/10') to 80.9% ('SMH 8177'). The average *Fusarium* kernel damage correlated significantly with the logarithm of average FHB index (r = 0.768) (Fig. 2).

Variables	Type I resist. (#IP)[1]	Type II resist. (#IS)[1]	Type I+II combined	FHBi (%)	FDK (%)	ERG (mg/kg)	DON (mg/kg)	ZEA (µg/kg)
Type II resist. (#IS)	0,012							
Type I+II combined	0,763**	0,643**						
FHBi (%)	0,403**	0,537**	0,655**					
FDK (%)	0,256	0,532**	0,526**	0,773**				
ERG (mg/kg)	0,295	0,511**	0,542**	0,771**	0,819**			
DON (mg/kg)	0,218	0,428**	0,440**	0,657**	0,765**	0,848**		
ZEA (µg/kg)	0,237	0,334*	0,364*	0,565**	0,541**	0,699**	0,585**	
DON+ ZEA stnd[2]	0,279	0,344*	0,410**	0,667**	0,626**	0,784**	0,816**	0,792**

Table 2. Coefficients of correlation between resistances of type I and II, Fusarium head blight index, *Fusarium* damaged kernels and accumulation of ergosterol and *Fusarium* toxins in grain of 61 lines of wheat inoculated with *F. culmorum* in experiments in Cerekwica and Radzików. [1] – coefficients for 42 lines; [2] – average from standardized concentrations of DON and ZEA. Variables log transformed. Coefficients significant at: * - P<0.05, ** - P<0.01

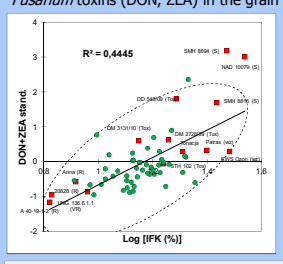
Contents of the ergosterol in grain of 61 selected lines amounted to an average of 30.9 mg/kg (Tab. 1). The range of variation was from 8.1 to 79.5 mg/kg. In the samples of grain from Cerekwica the contents of the ERG was 3 times higher (47.3 mg/kg) than in samples from Radzików (14.6 mg/kg).

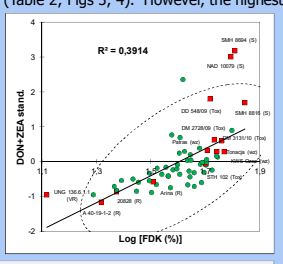
An average content of DON (including derivatives) in the grain of winter wheat was 20.515 mg/kg (Tab. 1). The DON content ranged from 4.055 mg/kg ('STH 9059') to 58.654 mg/kg ('NAD 11100'). In the samples of grain from Cerekwica the contents of the DON was 20 times greater (38.724 mg/kg) than in samples from Radzików (2.306 mg/kg). In the grain of wheat there was no presence of the derivative of a DON - 3AcDON and traces of 15AcDON and nivalenol.

The average content of zearalenone in the grain of winter wheat was 932 mg/kg (Tab. 1). In samples from Radzików only traces of ZEA were found (below the limit of quantification), and therefore they were not included in the tables. The content of ZEA was in the range from 48 mg/kg ('STH 1144') to 6450 mg/kg ('NAD 10079' S).

The ERG/DON ratio was calculated indicating the amount of ergosterol (mycelium amount) necessary to produce 1 mg of DON. The average value of the ratio was 1.51 mg ERG/mg DON. The coefficient varied between 0.59 ('NAD 11100') to 2.37 ('POB 0212'). Genotypes with low contents of DON in the grain (<15 mg/kg) had varied ERG/DON values, e.g. >2: 'A 40-19-1-2' (R), 'STH 9007', 'KBP 1040', 'POB 0212', 'STH 032' or <1,5: 'STH 1144', 'KBP 04.164', 'NAD 11017'. Also among the lines which accumulated a larger quantity of DON lines with ERG/DON above 2 were found: 'KWS Ozon' (wz), 'DD 548/09' (Tox). High value of the ratio indicates a higher resistance of type V.

Fusarium head blight index (FHBi) and Fusarium damaged kernels (FDK) correlated significantly with the contents of *Fusarium* toxins (DON, ZEA) in the grain (Table 2; Figs 3, 4). However, the highest were values of correlation coefficients of FDK versus ergosterol (ERG) and ERG content versus DON accumulation in the grain (tab. 2, Fig. 5, 6). The coefficient of correlation ERG versus ZEA accumulation in the grain was lower (Fig. 7). Resistance of type I (to infection) for 42 lines correlated with FHB index only (Tab. 2). While resistance of type II (to *Fusarium* spread) correlated with FHB index and FDK as well as with ergosterol and toxin content in the grain. Type II resistance is associated with resistance to DON, which is an essential factor for pathogen spread in the tissues. The ability to produce it impacts the aggressiveness of given *Fusarium* isolate (*F. culmorum*, *F. graminearum* and other DON producers).





Figures 3-4. Relationship between Fusarium head blight index (FHBi) and *Fusarium* damaged kernels (FDK) and total accumulation of *Fusarium* toxins (DON, ZEA) in grain of 61 winter wheat lines. DON+ZEA = average of standardized contents of DON and ZEA. Check lines/cultivars were marked with squares.

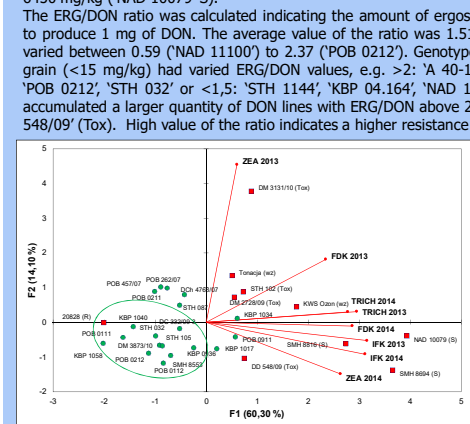
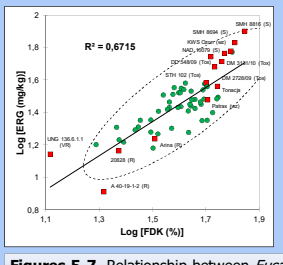
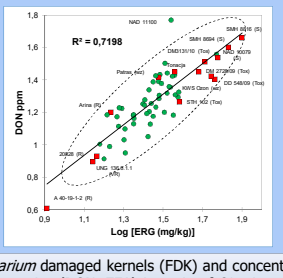
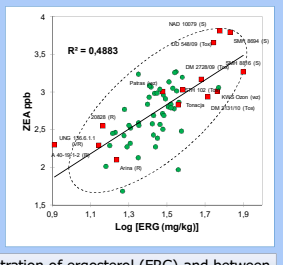


Figure 6. Biplot of the principal component analysis for 29 winter wheat lines. Two first components explained 74.4% of variability of FHB resistance characterized with FHB index (IFK), *Fusarium* damaged kernels (FDK) and concentration of zearalenone (ZEA) and trichothecenes B (TRICH) in grain in 2013 and 2014 in Radzików and Cerekwica

Among the studied lines, 29 accessions were also evaluated in the infection experiments in 2013 in Radzików and Cerekwica. FHB indexes, the percentage of *Fusarium* damaged kernels and the accumulation of trichothecenes B in grain in both years correlated significantly (r = 0.878, r = 0.448, r = 0.525, respectively). There was no correlation of the accumulation of zearalenone in both years. The highest resistance of all types showed the resistant line '20828' (R) and lines 'KBP 10 40', 'KBP 10 58' and 'POB 0111'. The remaining resistant lines were shown on the Figure 8.







Figures 5-7. Relationship between *Fusarium* damaged kernels (FDK) and concentration of ergosterol (ERG) and between concentration of ergosterol and *Fusarium* toxins (DON, ZEA) in grain of 61 winter wheat lines. Check lines/cultivars were marked with squares.

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