Article

Resistance to Fusarium head blight, kernel damage and concentration of *Fusarium* mycotoxins in grain of winter wheat lines

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Abstract: Fusarium head blight (FHB) can cause contamination of cereal grain with mycotoxins. Winter wheat is also infected with FHB. It is more resistant than durum wheat to head infection and less than other small grain cereals. The aim of this study was to identify winter wheat lines that combine low head infection and kernel damage with low toxin contamination. Resistance to FHB of 27 winter wheat lines and cultivars was evaluated over a three-year experiment established in two locations. At the anthesis stage, heads were inoculated with *Fusarium culmorum* isolates. The FHB index was scored and the percentage of *Fusarium*-damaged kernels (FDKs) assessed. The grain was analysed for type B trichothecenes (deoxynivalenol and derivatives, nivalenol) and zearalenone content. The average FHB index was 12.9%. The proportion of FDK was 6.9% (weight) and 8.5% (number). An average content of deoxynivalenol amounted to 3.543 mg/kg and nivalenol to 2.115 mg/kg. In total, it was 5.804 m/kg of type B trichothecenes. The zearalenone content in the grain was 0.214 mg/kg. Relationships between FHB index, FDK and mycotoxin contents were highly significant for wheat lines; however, they were stronger for FDK versus mycotoxins. Breeding lines combing all types of FHB resistance were found, and five of them had resistance similar to that of wheat lines with the *Fhb1* gene.

Keywords: winter wheat, mycotoxins, *Fusarium*, resistance, ergosterol, trichothecenes, zearalenone

Key Contribution: Identification of breeding lines of winter wheat resistant to FHB and accumulation of type B trichothecenes in grain. Lines showed resistance in different environments and do not carry major FHB resistance genes e.g *Fhb1*.

1. Introduction

Mycotoxins are widely studied in small grains cereal worldwide. As a secondary metabolites they are produced by fungal strains mostly by *Fusarium*, *Aspergillus* and *Penicillium* species. In case of wheat the mycotoxins as nivalenol, deoxynivalenol and also zearalenon are mostly founded. They are produced by pathogenic fungi *Fusarium graminearum* and *F. culmorum* and occurred not only in grains but also in wheat based product, what has detrimental effect on human and animal health [1,2].

Fungal diseases of bread wheat are one of the main factors that can lead to a decrease in grain yield and a decrease of grain quality. An important group of fungi with a parasitic-saprotrophic lifestyle and causing several cereal diseases, including wheat, are the species from genus *Fusarium*. They are the group of the most important pathogens of wheat [3–6]. Fusarium head blight (FHB) of wheat caused billions of dollars in losses for both farmers and grain marketing and processing companies [6–9]

In world literature, the most information on the Fusarium head blight of cereals relates to widely grown wheat [6,10,11]. FHB causes the greatest damage to bread wheat and durum wheat and the last one is the most susceptible to this disease compared to other cereals [12]. It is referred to as a disease complex because it is caused by several species of *Fusarium*. The favorable factor for the epidemic of FHB is windy weather and rainfall, especially during anthesis. The increase in share of maize and winter wheat in

cultivation, combined with simplified crop rotation, the change in cultivation techniques (zero tillage) and climate change have increased the risk of this disease over the last years [13–16]. Literature reports indicate that this problem is worldwide. Many years of research of winter wheat varieties in Poland show a small variation in susceptibility to FHB and indicate a lack among Polish cultivars genotypes with a low content of *Fusarium* mycotoxins in the grain [17–20].

Fusarium infection leads to the development of FHB which appears on cereal spikes, where through one spikelet the pathogen can infect the entire spike [21]. FHB is characterized by altered spike morphology like brownish stains with spikelets bleaching and drying up of kernels as a symptoms of spike premature dieback [22]. Additionally on the spike's glumes can be visible pink-red sporodochium, which are the source of fungi spores and can be the infection threat for the neighboring plants [23].

FHB severity is characterized by serval resistance types: resistance to initial infection (type I), resistance to *Fusarium* spread within the spike (type II), resistance to kernel infection (type III), tolerance against FHB and trichothecenes (type IV) and resistance to trichothecenes (type V) by degradation or detoxification (class 1) or by preventing of accumulation (class 2) [10,24].

Fungi of the genus *Fusarium* produce numerous toxins that can be highly or chronically toxic to both humans and animals, depending on the type of toxin and the amount of food or feed consumed [25]. Analysis of toxins in wheat kernels shown presence of predominantly two toxins types, type B trichothecenes like nivalenol (NIV) and deoxynivalenol (DON), and the second type of zearalenon (ZEN) [4,26,27].

Trichotecenes (DON, NIV) has a strong toxic effect, causing skin irritation, vomiting, diarrhea, appetite weakness, hemorrhages, neurological disorders, miscarriages and may even lead to death [28–30]. As stated by World Health Organization deoxynivalenol is consider as a teratogen, neurotoxin and immunosuppressive agent. DON together with NIV can cause diarrhea [31], nausea and food refusing in nonruminant animals [32]. The toxicity of zearalenon emerge on swine reproductivity disturbance due to demonstration of hyper estrogenic syndrome which cause genitalia and mammary glands enlargement and even livestock infertility [33–35].

To control and reduce the mycotoxin content of wheat and other cereal grains, limits have been set on the content of *Fusarium* toxins in food and feed in many countries, including the European Union. These limits are governed by the most recent Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 fixing maximum levels for certain contaminants in foodstuffs in respect of *Fusarium* toxins in maize and maize products.

The way to reduce the content of *Fusarium* toxins may be to reduce the risk of infestation of wheat crops by *Fusarium* through the use of fungicide and agrotechnical methods including: the use of ploughing, crop rotation (reduction of the share of cereals and maize), the cultivation of resistant cultivars [36,37].

The most effective method to counteract *Fusarium* infection is the breeding of genetically improved cultivars with resistance to the pathogen. Resistance to FHB is a quantitative trait. There are known many minor genes in *Triticum aestivum* genome which display additive effect [38], if they are inherited polygenically [39] as a Quantitative Traits Loci (QTL). The main QTL is the well examined *Fhb1* gene, located on the short arm of chromosome 3B, which increase the type II resistance in wheat [40,41]. Presence of this gene resistant allele can reduce about 20% of FHB severity [42]. Also it was noticed that *Fhb1* is connected with plant detoxification due to conversion of DON to less toxic DON-3-*O*-glucoside [43]. Out of large number of reported QTL's [44], some of them also contributed to increasing of plant resistance: *Fhb2* [45], *Fhb4* [46], *Fhb5* [47], *Qfhs.ifa-5A* [48].

Majority of resistant germplasm originated from China, from area where numerous FHB epidemics have been occurred [49]. Cultivar 'Sumai-3' and its consecutive derivatives, have been the most used in breeding programs, what leads to elevation of plant resistant level [50,51]. Despite the intensive screening for resistant germplasm, the genotypes with full resistance to FHB have not been found [8]. As we know, the environmental

conditions like high temperature and humidity foster *Fusarium* infection, what is important in case of climate change [52]. The environmental changes can influence on *Fusarium* species distribution with the pathogenic species shifts and its expansion on greater area [53,54]. Also some of *F. graminearum* isolates can have more intensely respond to the elevated temperature and CO_2 concentration, due to synthesis of higher amount of DON and ZEA toxins [35]. This represents a huge threat for future breeding of wheat, so it's important to looking for a new resistant cultivars.

The aim of the work was to find winter wheat genotypes resistant to FHB by assessing the degree of head infection and damage to kernels by *Fusarium culmorum*. Selected genotypes with increased FHB resistance were tested for ergosterol (ERG), quantitative meter of mycelium content and resistance to accumulation of *Fusarium* toxins — DON and derivatives, NIV and ZEN in the grain. These analyses were conducted to identify genotypes combining elevated levels of resistance to FHB, kernel damage and mycotoxin accumulation in the grain.

2. Materials and Methods

2.1 Plant material

Plant material comprised 27 winter wheat lines and cultivars:

- Winter wheat cultivars: 'Arina', 'Artist', 'Fregata', 'Patras', 'RGT Kilimanjaro' [55]. 'Artist', 'Patras', and 'RGT Kilimanjaro' were check cultivars in pre-registration testing system of the Research Centre for Cultivar Testing - COBORU (https://coboru.gov.pl) [56].
- Polish breeding lines of wheat: 'AND 4023/14', 'AND 82/11/50', 'DL325/11/3', 'KBP 14 16', 'NAD 10079', 'NAD 13014', 'NAD 13017', 'NAD 13024', 'POB 0616', 'POB 170/04', 'POB 679/03', 'SMH 7983', 'SMH 8694', 'SMH 8816', 'STH 008', and 'STH 9059'.
- Lines of wheat resistant to FHB carrying *Fhb1* gene: 'UNG 136.6.1.1 [Fhb1+]', 'S10 [Fhb1+]', S30 [Fhb1+] and 'S32 [FHb1 +]' [57,58].
- Lines of wheat resistant to FHB without *Fhb1* gene: '20828', 'A40-19-1-2' [57,59,60]

Wheat lines originated from Polish breeding companies (DANKO Hodowla Roślin Ltd., Choryń, Poland; Hodowla Roślin Smolice Ltd. – IHAR-PIB Group, Smolice, Poland; Hodowla Roślin Strzelce Ltd. – IHAR-PIB Group, Strzelce, Poland; Małopolska Hodowla Roślin Ltd., Kraków, Poland; Poznańska Hodowla Roślin Ltd., Tulce, Poland) and were selected from the large set of breeding lines based on low or high head infection in two environments (data not shown) [61].

2.2 Fungal material for inoculation

The fungal material for inoculation was a mixture of three isolates of *Fusarium culmorum* (W.G.Sacc.): KF 846 (DON chemotype), KF 350 (NIV chemotype) derived from the collection of Institute of Plant Genetics Polish Academy of Sciences (Poznań, Poland) and ZFR 112 (DON chemotype, producing zearalenone) derived from the collection of Plant Breeding and Acclimatization Institute - NRI (Radzików, Poland) [62].

Isolates were incubated on autoclaved wheat kernels in glass flasks for about 1 week at 20°C in darkness and next exposed to near UV light under a 16-h photoperiod for 3 weeks at 15°C. The mycelium-colonized grain was air dried and stored in a refrigerator at 4°C until usage. At the date of inoculation, the grain with *F. culmorum* spores was suspended in water for about 2 h and then filtered through cheesecloth to obtain a conidial suspension. The suspensions from each of the three isolates were adjusted to 500 000 spores/ml with the aid of a haemocytometer (BRAND GmbH + Co. KG., Wertheim, Germany). Equal volumes of suspension from the three isolates were mixed.

2.3 Description of the field experiment

A three-year field experiment (2017, 2018 and 2019) was established in two locations. First was experimental field of Institute of Plant Genetics Polish Academy of Sciences in Cerekwica (30 km north-west from Poznań, Poland; 82 m above sea level; GPS coordinates

52°31'21.3"N 16°41'19.1"E). Second was experimental field of Plant Breeding and Acclimatization Institute National Research Institute in Radzików (central Poland; 87 m above sea level; GPS coordinates 52°12'45,4"N 20°37'59.2"E).

Experiments were established as a randomized block design. Wheat lines were sown in 1 m^2 (Radzików) or 0.5 m^2 (Cerekwica) plots in four replicates/blocks. Sowing dates were within the range from the last week of September to the first week of October. Conventional tillage was applied in both locations.

2.4 Inoculation procedure

At full anthesis (65 BBCH scale) in end of May to 10 day period of June wheat lines were inoculated by spraying heads with spore suspension [63]. Three blocks of plots were inoculated and fourth non-inoculated served as a control. Inoculation was repeated three days later. During two days after inoculation micro-irrigation was applied to maintain high moisture level [62,64].

Three weeks after inoculation disease progress was visually evaluated as the Fusarium head blight index (FHBi):

$$\text{FHBi} = \frac{\%\ \text{of head infection} \times \%\ \text{of heads infected per plot}}{100} \quad (1)$$

At the harvest, 20 randomly selected heads from each plot (one control and three inoculated plots) in each location were collected and threshed with laboratory thresher.

The percentage of *Fusarium* damaged kernels (FDK) was scored visually according to the methods described earlier [65,66]. FDK weight in relation to the weight of whole sample was marked as FDKw and FDK number in relation to the total sample size was marked as FDK#.

2.5 Toxins analysis

Wheat grain samples were fine ground. The concentration of *Fusarium* toxins in wheat grain was analysed using the technique of gas chromatography. The type B trichothecenes (DON, 3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON) and NIV) were detected. The methodology used for the extraction and detection of the samples with use of gas chromatography was described in detail by Góral et al. [67,68].

The content of ZEN was determined using a quantitative direct, competitive enzyme-linked immunosorbent assay (ELISA) AgraQuant® Zearalenone 25-1000 (LOD 20 ppb, LOQ 25 ppb) (Romer Labs GmbH, Tulln, Austria). The detailed methodology used for the quantitative analysis of ZEN was described by Góral et al. [67].

Ergosterol was chromatographically analyzed using HPLC technique on a silica column using methanol. Detection was carried out on the UV detector. Detailed procedure was described by Góral et al. [69]

2.6 Statistical analysis

The statistical analysis was performed using XLSTAT© Life Science, Version 2021.2.1.1119 (Addinsoft, New York, USA).

FHB and FDK ratings, reduction of yield components and concentration of ERG and toxins data were analyzed by analysis of variance procedures using the XLSTAT procedure: ANOVA. Year effect was considered random and location and line were considered fixed. Normality of data distribution was tested with the Shapiro-Wilk test (XLSTAT procedure: Normality test). All variables did not follow normal distribution and were transformed with Box-Cox (FHBi, FDKw, FDK#) or log10 (ERG, DON, 3ADON, 15AcDON, NIV, TCT B, ZEN) transformations.

The relationships between FHBi, FDK, ERG and mycotoxin concentrations were investigated by Pearson correlation tests (XLSTAT procedure: Correlation tests). Prior to analysis variables (means for 27 lines) which did not follow normal distribution were log10 transformed to normalize residual distributions.

Multivariate data analysis method was applied to the data on FHB resistance (FHBi, FDK#, ERG, DON, NIV, ZEN). Principal component analysis (XLSTAT procedure:

Principal Component Analysis PCA) was used to show how wheat lines are distributed with respect to the main variation described in the first two components and how variables influenced the construction of the two components. PCA results also revealed associations among variables measured by the angle between variable vectors.

3. Results

The average severity of FHB was FHBi = 12.9%. It was higher in Radzików (15.2%) than in Poznań (9.4%) (Figure 1). The range of reactions was from 0 to 64.0% in Radzików and from 0 to 54.0% in Cerekwica. Proportion of *Fusarium* damaged kernels was on average FDK w = 6.9% and FDK # = 8.5%. It was higher in Poznań (FDKw = 9.8%; FDK# = 12.0%) than in Radzików (FDKw = 5.0%; FDK# = 6.2%). The range of reaction was from 0 to 61.2% in Radzików and from 0 to 45.8% in Cerekwica for FDK w and from 0 to 62.1% in Radzików and from 0.1 to 61.5% in Cerekwica for FDK #.

In three experimental years, FHB index amounted to 17.2% in 2017, 7.8% in 2018 and 13.7% in 2019. As regards FDK proportion, it was 11.5% and 13.2% in 2017, 6.6% and 8.3% in 2018, 2.6% and 4.1% in 2019, FDK w and FDK #, respectively.

Concentration of ERG in grain was on average 11.6 mg/kg. It was higher in samples form Cerekwica (Figure 2). Concentration range was 0.5 – 49.5 mg/kg in Cerekwica and 1.7 – 72.6 mg/kg in Radzików. In 2017 average ERG content in grain was 18.4 mg/kg, in 2018 10.5 mg/kg and in 2019 5.8 mg/kg.

Amount of DON in grain was on average 3.543 mg/kg at a range 0 – 25.960 mg/kg and amount of NIV in grain was 2.115 mg/kg at the range 0 – 17.400 mg/kg. Concentrations of DON in Cerekwica and Radzików (Figure 2). On the contrary, concentration of NIV in Radzików was very low (0.485 mg/kg) and about 7-times lower than in Cerekwica (3.756 mg/kg). In three experimental year amounts of DON and NV were as follows: 2017 – 6.846 and 3.436 mg/kg, 2018 – 1.622 and 2.491 mg/kg, 2019 – 2.162 and 0.418 mg/kg.

Acetylated derivatives of DON (3AcDON, 15AcDON) were detected at low amounts. On average it was 0.130 mg/kg of 3AcDON (0 – 1.375 mg/kg) and 0.016 mg/kg of 15AcDON (0 – 0.325 mg/kg). In Cerekwica concentration of 3AcDON and 15AcDON was 0.145 and 0.017 mg/kg respectively and in Radzików 0.114 and 0.015 mg/kg. In three experimental year amounts of 3AcDON and 15AcDON were as follows: 2017 – 0.275 and 0 mg/kg. 2018 – 0.024 and 0.048 mg/kg, 2019 – 0.090 and 0 mg/kg.

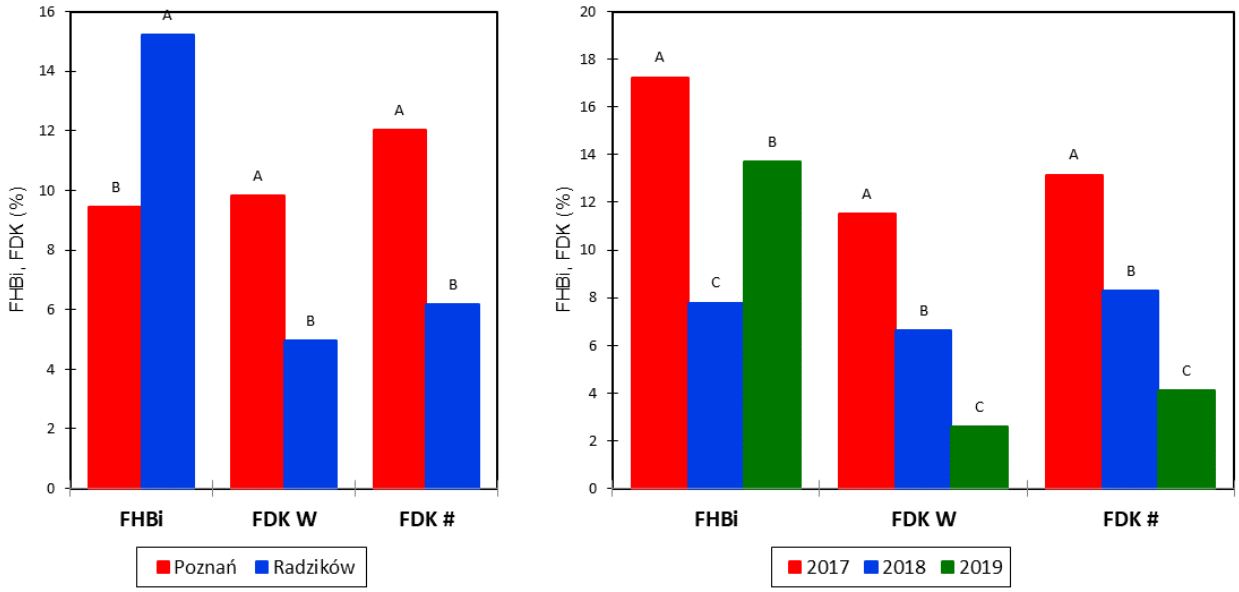

Figure 1. Average Fusarium head blight index (FHBi) and *Fusarium* damaged kernels percentage (FDK# - number, FDK w – weight) in two experimental locations (left) and three experimental years (right). Means marked with the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test performed on transformed variables.

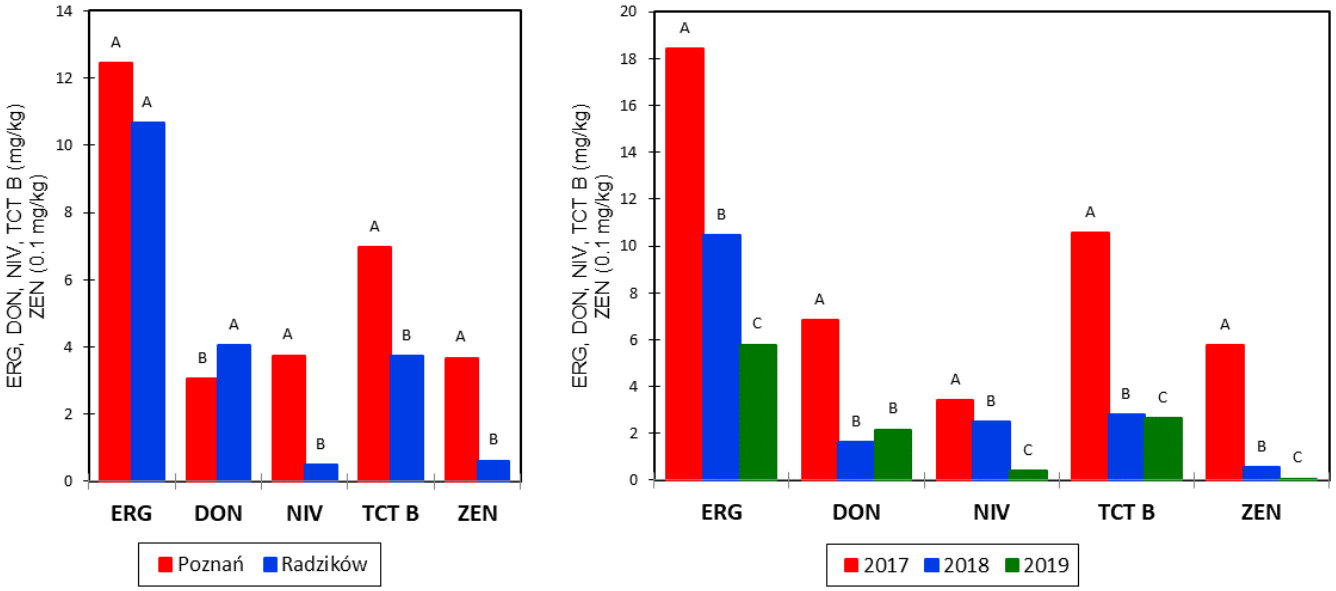

Figure 2. Average concentration of ergosterol (ERG), deoxynivalenol (DON), nivalenol (NIV), type B trichothecenes (TCT B) and zearalenone (ZEN) in grain of 27 wheat lines in two experimental locations (left) and three years (right). 3AcDON and 15AcDON were not shown. Means marked with the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test performed on transformed variables.

Total amount of analyzed type B trichothecenes was 5.804 mg/kg at a range 0 – 30.854 mg/kg. In Cerekwica amount of TCT B was 6.962 mg/kg at a range 0.143 – 29.900 mg/kg and in Radzików 4.1646 mg/kg at a range 0 – 30.5845 mg/kg. In three years, average amount of TCT B was as follows: 2017 – 10.557 mg/kg, 2018 – 4.7185 mg/kg, 2019 – 2.671 mg/kg.

Zearalenone was detected in grain at average amount of 0.214 mg/kg. The amount range was from 0 to 3.714 mg/kg. ZEN was present mainly in samples from Cerekwica at amount of 0.367 mg/kg. In samples from Radzików its concentration was 5 times lower and amounted to 0.061 mg/kg. The highest concentration of ZEN was detected in 2017 at 0.578 mg/kg, followed by 2018 at 0.057 mg/kg. In 2019 it was very low at 0.008 mg/kg.

Table 1. Analysis of variance of Fusarium head blight index and *Fusarium* damaged kernels percentage (weight, number) for 27 wheat cultivars and lines

Source	DF	FHBi		FDKw		FDK#	
		Mean squares	F	Mean squares	F	Mean squares	F
Year	2	42.854	1.776	33.213	9.005	56.314	10.282
Location	1	27.028	1.107	11.014	2.971	18.137	6.615*
Line	26	13.845	17.868***	2.521	9.787***	84.079	11.324***
Year x Location	2	24.419	23.049***	3.707	13.442***	5.484	9.488***
Year x Line	52	0.775	0.731	0.258	0.934	14.850	0.988
Location x Line	26	2.120	2.001*	0.186	0.676	6.037	0.803
Year x Location x Line	52	1.059	3.867***	0.276	2.656***	15.027	2.166***
Error	234	0.274		0.104		32.427	

***, * significant at P < 0.001 and 0.05

Analysis of variance of FHB index showed very high effect of wheat line and no effect of year (random) and location (Table 1). No interaction year x line was observed. Highly significant interactions year x location and year x location x line were found. Similarly, for FDKw and FDK# effect of line was highly significant as well year x location and year x

location x line interactions. Interactions location x line were not significant for FDK's. For FDK # effect of location was low significant.

We found highly significant effect of year on concentration of all analysed toxins (Tables 2, 3). Location had highly significant effect on concentration of NIV and sum of trichothecenes but only weak on DON and ZEN concentration.

Table 2. Analysis of variance of concentration of ergosterol, DON, 3ACDON and 15AcDON in grain of 27 wheat cultivars and lines

Source	DF	ERG		DON		3AcDON		15AcDON	
		MS	F	MS	F	MS	F	MS	F
Year	2	2.544	56.128***	3.829	72.491***	0.113	62.108***	0.007	21.388***
Location	1	0.076	1.667	0.239	4.517*	0.007	3.813	0.000	0.082
Line	26	0.290	6.397***	0.247	4.675***	0.008	4.410***	0.000	0.745
Error	132	0.045		0.053		0.002		0.000	

***, * significant at P < 0.001 and 0.05

Location mean squares for NIV was higher than year mean squares. This toxin was mainly detected in samples from Poznań (NIV) (Figure 2). Effect of wheat line was highly significant for ERG and trichothecenes (except 15AcDON) and medium significant for ZEN.

Table 3. Analysis of variance of concentration of nivalenol, trichothecenes B (sum of DON, 3AcDON, 15AcDON and NIV) and zearalenone in grain of 27 wheat cultivars and lines

Source	DF	NIV		TCT B		ZEN	
		MS	F	MS	F	MS	F
Year	2	2.003	53.983***	4.026	117.699***	47.632	74.966***
Location	1	6.629	178.702***	1.540	45.029***	4.272	6.723*
Line	26	0.136	3.672***	0.353	10.319***	1.297	2.041**
Error	132	0.037		0.034		0.635	

***, **, * significant at P < 0.001, 0.01 and 0.05

Winter wheat line 'KBP 14 16' showed the highest value of FHB index and the lowest FHBi was for lines carrying *Fhb1* resistance gene ('UNG 136.6.1.1 [Fhb1+]', 'S 10 [Fhb1+]', 'S 32 [Fhb1+]') and without *Fhb1* but resulted from crosses with resistant genotypes ('A40-19-1-2', '20828') (Table 4). Among breeding lines, the lowest FHBi was observed for four lines: 'SMH 7983', 'NAD 13014', 'NAD 13017', 'STH 9059'. Highly infected heads had three lines 'DL325/11/3', 'SMH 8694', and 'SMH 8816'.

The lowest FDK proportions (weight, number) were observed for low FHB infected lines carrying *Fhb1* gene, but also for line '20828'. Line 'A40-19-1-2' had higher FDK values. Among breeding lines, the lowest kernel damage was found for four lines 'POB 679/03', 'STH 9059', 'A40-19-1-2', 'POB 170/04' and 'NAD 13014'. FHB susceptible lines ('DL 325/11/3', 'KBP 14 16') showed also high FDK values. Two high yielding cultivars 'Artist' and 'Patras' had also high level of kernel damage. The third cultivar 'RGT Kilimanjaro', despite similar head infection, exhibited twice lower FDKs than two previous cultivars.

Table 4. Fusarium head blight index and *Fusarium* damaged kernels percentage (weight, number) for 27 winter wheat lines and cultivars

Line	FHBi (%)	FDKw (%)	FDK# (%)
KBP 14 16	41.6 a	25.2 a	28.7 a
DL325/11/3	37.0 ab	17.5 ab	20.4 ab
SMH 8694	35.5 ab	10.3 bc	12.9 bc
SMH 8816	32.8 ab	8.9 cd	11.0 cd
NAD 10079	28.7 abc	9.1 bc	11.2 bc

Patras	22.1 bcd	9.3 cd	13.3 cd
Artist	20.0 cde	10.2 bc	13.4 bc
RGT Kilimanjaro	14.4 def	5.0 defg	7.3 def
Arina	10.9 efgh	6.8 cde	8.0 cde
POB 679/03	10.1 fghi	4.4 efgh	4.0 ghijk
POB 0616	9.8 ghi	6.9 defg	8.4 efg
STH 008	9.7 fghi	7.2 defg	8.9 def
NAD 13024	8.9 efgh	5.8 efg	7.5 efg
AND 82/11/50	8.8 efg	6.7 cdef	9.3 cde
POB 170/04	8.6 ghi	4.6 efg	4.7 fghi
AND 4023/14	7.1 ghi	4.7 efgh	6.8 efg
SMH 7983	6.6 ghij	5.8 efgh	7.3 efgh
NAD 13014	5.6 ghij	4.1 fghi	5.3 efghi
Fregata	5.1 ghij	5.9 efg	7.7 efg
NAD 13017	4.9 hij	4.6 efgh	5.9 efghi
STH 9059	4.7 ij	3.9 ghij	4.6 fghij
S 30 [Fhb1+]	3.8 j	3.4 jk	4.2 kl
UNG 136.6.1.1 [Fhb1+]	2.9 j	3.6 ghijk	4.2 hijkl
S 10 [Fhb1+]	2.4 j	3.6 hijk	4.5 ijkl
S 32 [Fhb1+]	2.3 j	2.1 k	2.4 l
A40-19-1-2	2.2 j	4.2 ghijk	5.0 ghijk
20828	2.1 j	2.6 ijk	2.9 jkl
Means	12.9	6.9	8.5

Means marked with the same letter are not significantly different at $p < 0.05$ according to Fisher LSD test performed on Box-Cox transformed variables; means ranked by FHBi values.

Ergosterol concentration was the highest in grain of susceptible wheat lines 'KBP 14 16' and 'DL 325/11/3'. (Table 5). It was also high in grain of cultivars 'Artist' and 'Patras' which had high level of kernel damage. ERG content was also high in grain of 'RGT Kilimanjaro' cultivar which had low damage of kernels. The lowest ERG content was found in grain of lines with *Fhb1* gene 'S 10 [Fhb1+]' and 'S 30 [Fhb1+]' as well as in resistant lines 'A40-19-1-2' and '20828'. As regards breeding lines, the lowest ERG concentration was found in grain of 'STH 9095' and 'SMH 7983'.

Deoxynivalenol accumulated at the highest amount in grain of susceptible wheat lines ('DL 325/11/3', 'KBP 14 16'). Large amount of DON was also detected in grain of two other susceptible lines ('SMH8694', 'SMH 8816') and two check cultivars 'Artist' and 'Patras'. Amount of DON in grain of 'RGT Kilimanjaro' was twice lower. Lowest concentration of DON was detected in grain of five resistant check lines. It was higher only in grain of line 'UNG 136.6.1.1 [Fhb1+]'. In grain of breeding lines DON amount was lowest for two lines with the lowest ERG concentration – 'STH 9095' and 'SMH 7983'. Nivalenol was present mainly in grain of susceptible lines 'KBP 14 16' and 'SMH 8816' as well as in grain of cultivars 'Artist' and 'Patras'. It was lower in grain of 'DL 325/11/3' line. Similar NIV amounts were detected in grain of two cultivars ('RGT Kilimanjaro', 'Arina') and resistant lines 'UNG 136.6.1.1 [Fhb1+]' and '20828'. Lowest concentration of NIV was detected in grain of three resistant wheat lines with *Fhb1* gene and four breeding lines ('STH 008', 'POB 170/04', 'STH 9059', 'POB 679/03'). 3AcDON was detected mainly in susceptible wheat lines and cultivars which accumulated large amounts of DON and NIV. Concentration of 15AcDON in grain was very low.

Total amount of four type B trichothecenes was the highest in grain four susceptible lines and two cultivars 'Artist' and 'Patras'. It was the lowest in grain of three lines with *Fhb1* gene. It was also low in grain of three breeding lines 'SMH 7983', 'POB 679/03', 'STH 9059'.

Differences in ZEN concentration between wheat lines were of low significance (Table 6). The lowest amount of ZEN was found in grain of resistant lines 'S 32 [Fhb1+]', 'S 10

[Fhb1+]' and '20828'. Low amount of ZEN (below 0.100 mg/kg) was accumulated in grain of lines 'POB 0616', 'STH 008, 'AND 4023/14', 'POB 679/03' and cultivar 'Fregata'. The highest amount of ZEN (on average 0.851 mg/kg, maximum 3.714 mg/kg) was found in grain of susceptible line 'KBP 14 16'.

Table 5. Concentration of ergosterol (mg/kg), type B trichothecenes (DON, 3AcDON, 15AcDON, NIV, TCT B) (mg/kg) and zearalenone (mg/kg) in grain of 27 winter wheat cultivars and lines.

Line	ERG	DON	3Ac DON	15Ac DON	NIV	TCT B [a]	ZEN
KBP 14 16	30.8 a	9.344 ab	0.453 a	0.035	6.326 a	16.158 a	0.851 ab
DL325/11/3	22.7 ab	11.698 a	0.453 a	0.021	3.426 abc	15.598 a	0.581 a
Artist	22.8 abc	6.780 b-e	0.275 b	0.026	4.629 ab	11.710 abc	0.485 b-e
Patras	25.9 abc	5.910 c-g	0.232 bcd	0.021	4.983 ab	11.147 b-e	0.245 cde
SMH 8694	20.0 ab	6.181 abc	0.232 bc	0.030	3.876 abc	10.318 ab	0.421 a-d
SMH 8816	17.1 ab	5.597 a-d	0.189 b-e	0.043	4.348 abc	10.177 ab	0.335 a-d
NAD 10079	16.3 a-e	4.772 a-d	0.122 b-g	0.024	3.959 abc	8.877 bcd	0.267 abc
AND 82/11/50	11.3 c-h	4.228 c-g	0.153 b-f	0.000	2.252 b-e	6.633 d-g	0.141 cde
RGT Kilimanjaro	17.6 b-f	3.275 c-h	0.125 b-g	0.015	2.816 a-d	6.232 d-g	0.349 cde
Fregata	9.8 e-h	4.472 c-f	0.100 c-f	0.010	1.145 def	5.727 e-h	0.062 de
Arina	10.9 c-g	3.783 c-f	0.075 efg	0.008	1.638 c-f	5.503 c-f	0.276 cde
NAD 13017	9.0 d-h	2.858 c-h	0.118 b-g	0.000	1.359 def	4.335 f-i	0.152 de
NAD 13024	7.4 f-i	3.013 c-h	0.102 c-g	0.000	1.195 def	4.311 f-i	0.124 cde
NAD 13014	7.3 f-i	2.356 d-i	0.087 d-g	0.000	1.623 def	4.065 f-j	0.273 de
POB 0616	13.2 d-h	2.556 f-j	0.079 efg	0.008	1.308 def	3.951 f-k	0.108 cde
UNG 136.6.1.1 [Fhb1+]	6.9 ghi	1.678 g-j	0.048 fg	0.008	1.960 def	3.695 h-l	0.108 de
AND 4023/14	5.7 ghi	2.092 e-j	0.092 d-g	0.000	1.332 def	3.516 f-k	0.085 cde
STH 008	7.7 f-i	2.478 c-h	0.114 c-g	0.008	0.861 ef	3.461 f-j	0.092 de
POB 170/04	6.4 ghi	2.224 d-i	0.092 d-g	0.021	0.861 ef	3.197 f-k	0.111 cde
SMH 7983	5.3 ghi	1.672 f-j	0.076 efg	0.000	1.136 def	2.883 g-k	0.193 de
POB 679/03	6.8 f-i	2.065 e-j	0.069 efg	0.029	0.610 ef	2.773 g-k	0.073 cde
A40-19-1-2	5.1 i	1.388 hij	0.033 fg	0.000	1.340 def	2.762 k-l	0.052 e
STH 9059	5.3 hi	1.705 f-j	0.073 efg	0.004	0.734 ef	2.516 i-l	0.218 cde
20828	5.5 i	1.031 hij	0.031 fg	0.049	1.393 def	2.504 jkl	0.047 e
S 30 [Fhb1+]	4.6 i	1.238 hij	0.034 fg	0.054	0.620 ef	1.946 kl	0.059 de
S 10 [Fhb1+]	4.5 i	0.561 j	0.017 g	0.000	0.912 ef	1.489 l	0.047 e
S 32 [Fhb1+]	6.2 hi	0.714 ij	0.024 fg	0.021	0.468 f	1.226 l	0.028 e
Means	11.6	3.543	0.129	0.016	2.115	5.804	0.214

[a] sum of DON, 3AcDON, 15AcDON and NIV; means marked with the same letter are not significantly different at $p < 0.05$ according to Fisher LSD test performed on $\log_{10}$ transformed variables; means ranked by TCT B concentration

Fusarium head blight index correlated significantly with other variables except 15AcDON (Table 6). *Fusarium* damaged kernel proportions (weight, number) correlated highly significantly with concentration of mycotoxins. The lowest values had coefficients of correlations with NIV. Ergosterol concentration in grain correlated significantly with all mycotoxins except 15AcDON. The lowest values had coefficients of correlations with ZEN. Trichothecene toxins correlated significantly which each other (except 15AcDON) and with ZEN. The lowest value had coefficient of correlation DON vs NIV.

Table 6. Coefficients of correlation between Fusarium head blight index, *Fusarium* damaged kernels proportion (weight, number) and concentration of ergosterol and mycotoxins in grain of 27 winter wheat lines.

Variables	FHBi	FDK w	FDK #	ERG	DON	3Ac DON	15Ac DON	NIV	TCT B

FDK w	0.894								
FDK #	0.884	0.984							
ERG	0.883	0.853	0.868						
DON	0.900	0.919	0.924	0.911					
3AcDON	0.837	0.922	0.908	0.854	0.911				
15AcDON	0.319 ^{ns}	0.177 ^{ns}	0.113 ^{ns}	0.291 ^{ns}	0.201 ^{ns}	0.256 ^{ns}			
NIV	0.802	0.797	0.827	0.902	0.826	0.796	0.291 ^{ns}		
TCT B	0.895	0.907	0.923	0.941	0.978	0.900	0.233 ^{ns}	0.924	
ZEN	0.840	0.810	0.818	0.798	0.833	0.807	0.111 ^{ns}	0.806	0.855

Coefficients significant at $P < 0.001$ except marked with ^{ns} - non-significant.

Multivariate PCA analysis showed that the highest FHB resistance described with six traits (FHBi, FDK#, ERG, DON, NIV, and ZEN) was found in resistant wheat lines carrying *Fhb1* gene and two lines without this ('20828', 'A40-19-1-2') (Figure 3). Among breeding lines, five other lines had also considerable FHB resistance ('POB 679/03', 'POB 170/04', 'AND 4023/14', 'STH 9095', 'SMH 7983'). The most susceptible were lines 'DL 325/11/3' and 'KBP14 16'. They accumulated high amounts of DON and ZEN and had significant kernel damage.

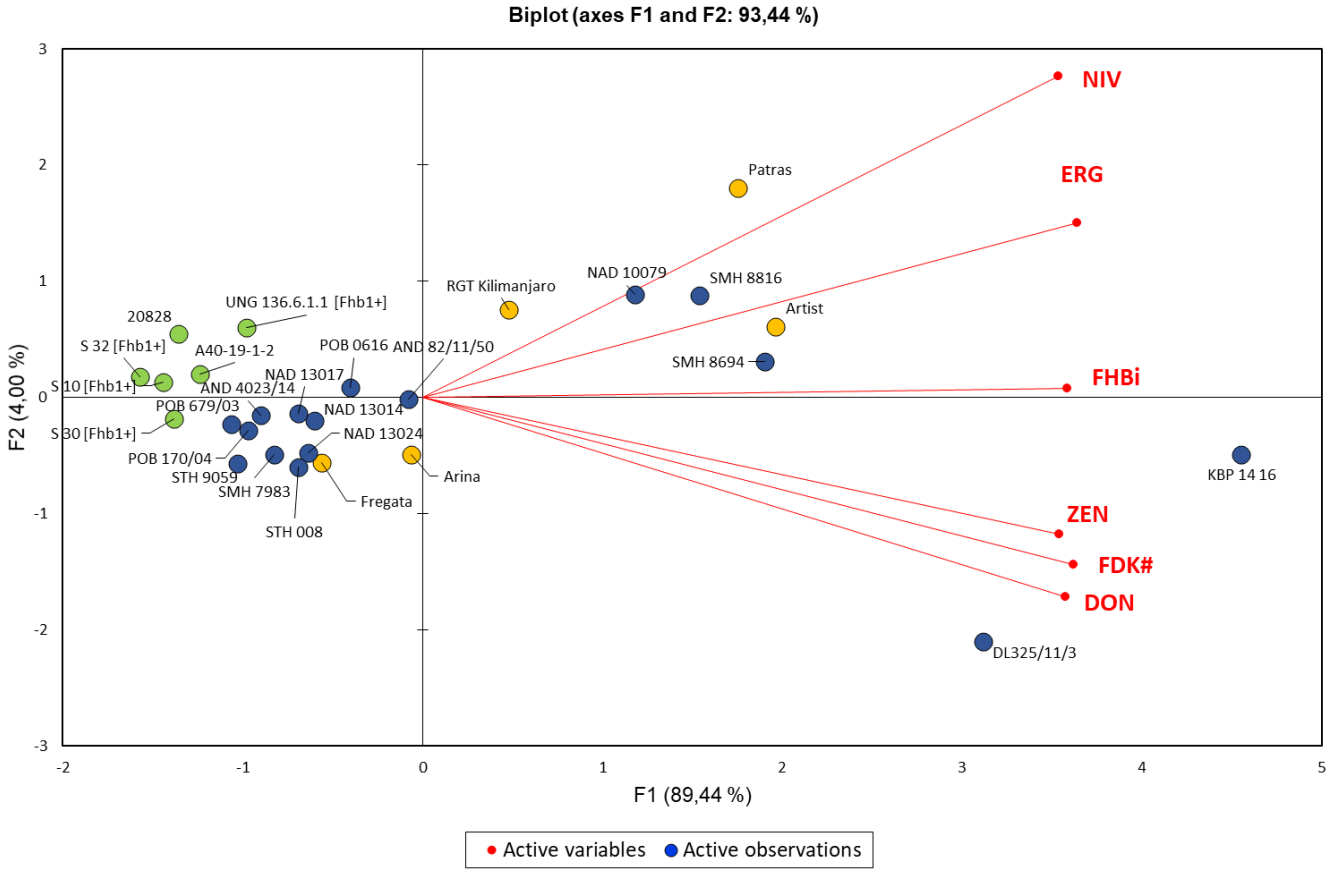

Figure 3. Biplot of principal component analysis (PCA) of FHBi, FDK#, and concentration of ERG, DON, NIV, and ZEN for 27 winter wheat lines and cultivars. FDK weight, 3AcDON and 15AcDON were not included. Highly resistant lines – green circles, breeding lines – blue circles, cultivars – orange circles.

4. Discussion

Research has shown that environmental conditions significantly affect the development of FHB and the accumulation of toxins in the grain [13,70]. Fusarium head blight severity, kernel damage and concentration of *Fusarium* metabolites were significantly

affected by the experimental year and the location. This study on resistance to FHB and accumulation of *Fusarium* toxins was conducted over three years in two locations. To maintain humidity during inoculation and after inoculation, mist irrigation was used in Cerekwica. However, the infection of heads was higher in Radzików than in Cerekwica, but the other parameters examined (percentage of FDK, the amount of ERG and toxins: sum of type B trichothecenes and zearalenone) were higher in Cerekwica. The exception was DON concentration which was higher in Radzików.

In 2017, the weather in May was similar in both locations, and in June, rainfall in Radzików was twice as high as in Cerekwica. This led to a higher head infection in Radzików. In July, rainfall in Cerekwica was double that of Radzików. This caused high kernel damage in Cerekwica, twice higher than in Radzików. However, the amounts of type B trichothecenes in grain in both locations were similar. While amount of ZEN in grain in Cerekwica was very high (1.077 mg/kg) and very low in Radzików (0.079 mg/kg). We observed differences in the accumulation of DON and NIV in both locations. DON was mainly found in grain from Radzików (8.256 mg/kg versus 5.435 mg/kg in Cerekwica) and NIV mainly in samples from Cerekwica 5.786 mg/kg versus 1.087 mg/kg in Radzików).

Weather in 2018 was less favourable for FHB development. Rainfall in June was low compared to previous years. In both locations, head infection was low, even despite application of mist irrigation in Cerekwica. Kernel damage was lower than in previous years and twice lower in Radzików than in Cerekwica. The same was found for sum of type B trichothecenes, which was twice lower. As in 2017, we observed the opposite results for DON and NIV concentrations. Very low amount of DON in samples from Cerekwica and very low amount of NIV in samples from Radzików. ZEN concentration was 10 times lower than in 2017 and five times higher in Radzików than in Cerekwica.

Weather conditions in Poznań in 2019 were unfavorable for the development of FHB. In Cerekwica rainfall was very low in June. It was a period of wheat flowering and inoculations. In the second half of June there were very high temperatures reaching 39°C. The use of a mist irrigation system enabled the inoculation of the heads, but symptoms were low. At the beginning of June, the precipitation in Radzików was much higher, which allowed effective inoculation and the occurrence of symptoms of head infection (higher than in Cerekwica). In the third decade of June there was no rainfall and air temperatures were very high (up to 38°C). Weather conditions caused inhibition of the development of FHB and the formation of mycotoxins which concentration was the lowest in three years.

Observed differences in accumulation of DON and NIV in two locations were probably the result of competition between isolates of different chemotypes. Competition between *F. culmorum* and *F. graminearum* species was described by Van der Ohe and Miedaner [71]. One isolate of *F. graminearum* was of the NIV chemotype and this isolate showed similar pathogenicity to isolates of the DON chemotype. Mixture of DON+NIV chemotypes had stable pathogenicity (head infection) but varied in mycotoxin production in experimental environments. The NIV chemotype is generally considered less aggressive than DON (3ADON, 15ADON) chemotypes [59,60]. Results of our experiments showed that it could produce considerable amounts of NIV even in mixture with a more aggressive isolate of the 3ADON chemotype. However, it occurred mainly in Cerekwica, where application of mist irrigation created conditions more favourable for FHB development.

Wheat breeding lines showing low susceptibility to FHB were identified. The disease symptoms (on heads and kernels) were low and like the resistant checks (with or without *Fhb1* gene). However, as regards trichothecene toxins in grain, three lines with *Fhb1* gene showed the lowest accumulation. The best breeding lines accumulated more trichothecenes. The amount was like in two resistant checks without *Fhb1* gene – '20828' and 'A40-19-1-2'. The first one was progeny of crossing 'Capo' winter wheat cultivar with 'Sumai 3', however did not carry resistance gene *Fhb1* [57].

The most effective FHB resistance gene is *Fhb1* (*Qfhs.ndsu-3BS*), derived from the Sumai 3 cultivar, which in various studies has been able to explain between 16% and 60% variability in the spread of the pathogen in head tissue (type II resistance). This gene is

commonly used in resistance breeding. A molecular marker UMN10 closely linked with the *Fhb1* gene has been developed to reliably identify the presence of the gene in breeding materials [41]. However, the number of commercial cultivars with the *Fhb1* gene is limited. This gene is found mainly in varieties of spring wheat grown in the United States, Canada and China [72,73]. It is not found in European winter wheat cultivars. The only cultivar of winter with *Fhb1* 'Jaceo' was withdrawn from the market [74]. Currently, there is one cultivar of winter wheat with *Fhb1* is 'MS INTA 416' grown in Argentina [75].

The lack of success in introducing the *Fhb1* gene to intensive winter European cultivars may be due to the fact that, despite the use of marker-assisted selection (MAS), the presence of this gene has a negative impact on yield and quality characteristics [76,77]. Another factor hindering the introduction of the *Fhb1* gene (or others - *Fhb2*, *Fhb5* etc.) is the significant influence of the genetic background of the recipient genotype on the expression of this gene [50]. Genetic studies of the European winter wheat population have shown a lack of QTLs with a high effect associated with FHB resistance [78–81]. For example, no QTL in the *Fhb1* region was found which confirms lack of variation of FHB resistance at the *Fhb1* locus. However, in the above studies, a large number of low-effect QTLs have been identified on all wheat chromosomes. The presence of only low-effect QTLs hinders an effective strategy for pyramiding FHB resistance.

In addition morphological characteristics also have a large impact on the degree of wheat infection by FHB and results of QTL mapping studies. These characteristics are: plant height, length of peduncle, awn length, head compactness, anther extrusion/or retention. A strong link between the height of wheat plants and the severity of FHB is emphasized [82–84]. This is mainly due to differences in the microclimate at the level of heads in low and tall wheat canopy. In addition, however, a genetic link between FHB susceptibility and the presence of the dwarf genes *Rht1* (*Rht-B1*) and *Rht2* (*Rht-D1*) was found [85,86]. The mechanism of this phenomenon is not fully known. It is explained by the effect of these genes on type I resistance by reducing the anther extrusion. Retention of anthers in flowers increases susceptibility to infection and stimulates the growth of mycelium. Selecting wheat lines towards open flowering can increase resistance to primary infection. The recently described dwarf gene *Rht24*, which does not affect the type of wheat flowering may also be used [87,88].

5. Conclusions

Wide variability of reaction of winter wheat lines to Fusarium head blight was found. Visual observation of head infection and kernel damage were reliable indicator of *Fusarium* toxin accumulation. Lines combing all types of FHB resistance were identified. However lines with the highest FHB resistance accumulated more than lines with the *Fhb1* gene used as a highly resistant check.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Air temperature and rainfall in May, June, and July of 2017, 2018, and 2019 in two experimental locations.

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Data Availability Statement: Data supporting reported results can be found at https://data.mendeley.com/datasets/sp36ghdx9k/1.

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