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Uni- and Multivariate Approaches to Evaluating the Susceptibility of Wheat Hybrids to Fusarium Head Blight

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Abstract

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Fusarium head blight (FHB) is a disease of small grain cereals caused by *Fusarium* species. The pathogens affect spikes and kernels, resulting in reductions of yield and its quality. The present study was conducted to evaluate variation in the FHB resistance of wheat F_2 hybrids derived from 16 crosses between winter wheat cultivars of various origin and with different susceptibility to FHB. Plants were inoculated with a conidial suspension consisting of a mixture of *F. culmorum*, *F. graminearum* and *F. avenaceum* isolates. After harvest 1000-kernel weight, number of kernels per spike and kernel weight per spike were evaluated in inoculated and control plants. Disease symptoms were observed on kernels of infected plants and the percentage of Fusarium-damaged kernels (FDK) was calculated. The data were statistically evaluated using uni- and multivariate analyses. A significant influence of genotype and treatment on all observed characteristics was detected. Contrasts between control and inoculated plants showed that inoculation lowered the mean values of all the yield-related traits significantly (in the statistical sense). Results of uni- and multivariate analyses enabled us to find three cross combinations which exhibited a low FDK percentage and simultaneously a relatively low reduction of 1000-kernel weight after inoculation. They may be promising for breeding wheat with improved resistance to FHB.

Keywords: ANOVA; kernel damage; MANOVA; resistance to Fusarium head blight; winter wheat

Resistance to diseases is, apart from yield, the main selection criterion in breeding new cereal varieties. In small grain cereals, resistance to Fusarium head blight (FHB), caused by fungi of the genus *Fusarium*, is one of the most important characteristics. *F. culmorum* (W.G.Sm.) Sacc., *F. graminearum* Schwabe and *F. avenaceum* (Fries) Saccardo infect the spikes of wheat during flowering. The infection reduces grain yield and quality by contamination of the harvested grain with toxic fungal secondary metabolites, mainly nivalenol (NIV) and deoxynivalenol (DON) (Chełkowski *et al.* 2000; Bottalico & Perrone 2002; Mesterházy 2002; Mardi *et al.* 2005; Buerstmayr *et al.* 2009; Kosová *et al.* 2009; Tamburic-Ilincic *et al.* 2011; Marin *et al.* 2013). Modern high-yielding cereal cultivars are usually susceptible to FHB. The selection of genotypes resistant to the disease is difficult, because resistance to FHB is a quantitative trait modulated by genetic factors and environmental conditions (Miedaner 1997; Mesterházy *et al.* 1999; Miedaner *et al.* 2001; Snijders 2004; Ma *et al.* 2006; Gosman *et al.* 2007; Cowger *et al.* 2009; Warzecha *et al.* 2011). The level of resistance can be assessed after artificial inoculation by observing the FHB symptoms on spikes (resistance type I and II) and/or on kernels, including assessment of the rate of Fusarium-damaged kernels (FDK) and the reduction in yield-related traits (resistance

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type III and IV) (Mesterházy 1995; Buerstmayr *et al.* 2009; Warzecha *et al.* 2011). According to Mesterházy *et al.* (2015) "centre of the breeding work should move toward the FDK and DON as FHB symptoms are less reliable". The authors showed an association between FDK and DON concentration and stated that breeding for resistance against FHB is the most effective method to decrease contamination of kernels by mycotoxins.

It is important in plant breeding to distinguish such cross combinations that provide the opportunity to find genotypes with desirable traits, including resistance to diseases. In the case of quantitatively inherited traits, promising crosses can be chosen on the basis of the general combining ability effects of parental cultivars (e.g. Griffing 1956), but this approach requires the implementation of a number of crosses in the respective systems (e.g. diallel, line × tester), which is time-consuming and rarely used in wheat breeding.

For the assessment of type IV plant resistance to FHB, several characteristics must be considered simultaneously, such as the percentages of FDK and healthy-looking kernels (HLK) in grain samples, number of grains per spike, grain weight per spike and 1000-kernel weight in control and artificially infected plants. To select the best crosses, appropriate statistical analysis should be applied. In the present study a multivariate approach is proposed for the estimation of resistance to FHB of F_2 hybrids from several cross combinations of winter wheat.

MATERIAL AND METHODS

Winter wheat F_2 hybrids of sixteen cross combinations – C2106/12 (1), C2118/12 (2), C2128/13 (3), C2153/12 (4), C2211/12 (5), C2272/12 (6), KBP-F13/13 (7), KBP-F60/13 (8), KBP-F86/13 (9), KBP-F87/13 (10), KBP-F91/13 (11), KBP-F106/13 (12), KBP-F141/13 (13), KBP-F235/13 (14), KBP-F259/13 (15), KBP-31053/13 (16) – were examined under field conditions in 2014. Hybrids of each cross combination were sown in ten rows, 1 m long with 30 seeds in each row and 25 cm space between rows. The order of cross combinations in rows was determined randomly. Control rows were established in the same manner at a distance of 2.0 m from the plots designated for inoculation. This isolation was necessary to protect the plants against infection during inoculation.

Wheat heads were inoculated with a mixture of a conidial suspension of *Fusarium culmorum* (isolate KF846), *F. graminearum* (isolate KF 2870) and *F. avenaceum* (isolate KF 1337). Conidia concentration was adjusted to 5×10^4 ml^{-1}. Inoculation was performed at full anthesis (Zadoks scale 65) (for details see Wiśniewska *et al.* 2016). After inoculation the plants were micro-irrigated for two days to maintain moisture. At harvest, 10 randomly selected main heads were collected from each row and threshed manually. In the harvested samples, kernels were visually scored and divided into two categories: Fusarium-damaged kernels and healthy-looking kernels. FHB severity was estimated as the percentage of FDK. Furthermore, the number and weight of kernels per spike of infected (NKS_I and KWS_I) and control (NKS_C and KWS_C) plants were recorded, and 1000-kernel weight was calculated for infected (TKW_I) and control (TKW_C) plants.

Statistical analysis. Univariate two-way analysis of variance (ANOVA) was applied for the evaluation of hybrids in terms of yield-related traits in control and infected treatments. The data for the rate of FHB were processed by one-way analysis of variance, because in the control treatment the values of this trait were close to zero. It is much better to base the selection procedure on a few traits; then besides the individual traits, the relationships (usually linear) between them are also taken into account. Hence, multivariate analysis of variance (MANOVA), canonical variables and Mahalanobis distances were applied as the main tools for the evaluation of hybrids in terms of all the traits considered simultaneously (Caliński & Kaczmarek 1973; Morrison 1976). Differences between inoculated and control plants regarding individual traits and all traits treated simultaneously were formulated as contrasts and tested by the *F* statistic (Caliński & Kaczmarek 1973). Besides the formal statistical methods, it is helpful to give a geometric presentation of the relationship between hybrids. The distribution of hybrids on a plane was presented using canonical variables. The shortest Mahalanobis distances between hybrids were presented as a dendrite (Caliński 1982).

Additionally, hybrids were divided into homogeneous groups in such a way that the variability (sum of squares of deviations) within a group is as small as possible, while the variability between groups is maximized. We use a statistical test to verify the significance of the proposed grouping.

RESULTS

Mean values of the observed traits in inoculated and control plants are presented in Table 1. Inoculation

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with *Fusarium* caused a decrease in the values of all the observed yield-related traits. The percentage of Fusarium-damaged kernels in all cross combinations was relatively high, and ranged from 47.10% (hybrid No. 13) to 70.80% (hybrid No. 8).

A wide range of variation was exhibited in all of the studied characteristics, in both control and inoculated plants. Coefficients of variation (CV) were generally higher in inoculated than in control plants – for infected plants they were about 2–3 times higher than for those in the control.

The lowest coefficient of variation was found for 1000-kernel weight, which in the control varied from 5.84% (hybrid No. 6) to 24.06% (hybrid No. 13),

Table 1. Mean values, standard errors (SE) and coefficients of variation (CV) for traits observed in control and inoculated wheat plants

Hybrid No.	Control						Inoculated							
	1000-kernel weight (g)		kernel weight per spike (g)		No. of kernels per spike		1000-kernel weight (g)		kernel weight per spike (g)		No. of kernels per spike		Fusarium-damaged kernels (%)	
	mean (SE)	CV (%)	mean (SE)	CV (%)	mean (SE)	CV (%)	mean (SE)	CV (%)	mean (SE)	CV (%)	mean (SE)	CV (%)	mean (SE)	CV (%)
1	44.9 (1.34)	8.79	2.44 (0.39)	20.12	53.6 (5.50)	44.20	21.1 (2.03)	30.56	0.59 (0.07)	57.41	26.8 (2.42)	28.51	60.43 (4.25)	22.23
2	55.3 (1.21)	6.56	3.12 (0.17)	17.72	56.2 (2.78)	15.64	28.6 (2.25)	24.82	0.80 (0.10)	39.46	27.9 (2.26)	25.71	51.13 (3.64)	22.49
3	45.8 (2.75)	17.93	3.08 (0.29)	29.32	67.1 (3.92)	18.48	16.4 (1.64)	31.84	0.52 (0.06)	38.77	31.6 (2.30)	23.09	70.30 (5.20)	23.39
4	50.2 (1.32)	7.83	3.32 (0.24)	20.39	65.7 (3.03)	14.60	18.2 (1.65)	28.69	0.40 (0.12)	52.35	21.4 (2.28)	33.71	61.74 (3.07)	15.74
5	50.6 (1.12)	6.08	3.32 (0.21)	22.67	65.8 (4.58)	22.00	18.6 (2.56)	43.59	0.58 (0.11)	64.59	29.4 (3.44)	37.05	56.66 (4.71)	26.32
6	44.8 (0.87)	5.84	3.22 (0.19)	19.00	72.0 (4.22)	18.55	18.9 (1.77)	29.68	0.75 (0.10)	43.55	41.5 (4.92)	37.46	60.13 (5.89)	30.98
7	51.3 (1.44)	8.31	3.18 (0.23)	22.82	61.2 (3.26)	16.82	20.2 (1.49)	23.39	0.65 (0.08)	41.54	31.8 (2.90)	28.82	52.27 (3.17)	19.22
8	55.3 (2.42)	13.18	3.36 (0.22)	21.36	60.5 (2.67)	13.95	16.7 (2.17)	41.09	0.46 (0.10)	68.31	26.6 (3.06)	36.41	70.80 (4.49)	20.10
9	50.0 (1.03)	6.20	3.24 (0.20)	19.36	64.7 (3.53)	17.23	16.5 (1.98)	37.97	0.48 (0.06)	41.40	28.8 (1.70)	18.65	65.31 (6.28)	30.41
10	48.4 (0.93)	6.10	3.48 (0.22)	20.30	71.7 (3.96)	17.47	16.4 (1.33)	25.65	0.55 (0.05)	26.78	33.7 (1.98)	18.55	63.78 (3.25)	16.11
11	47.1 (1.38)	9.28	2.75 (0.12)	14.09	58.3 (1.91)	10.36	16.8 (1.53)	28.77	0.36 (0.06)	54.09	20.4 (2.38)	36.92	59.10 (3.60)	19.30
12	56.4 (3.39)	19.00	3.47 (0.21)	19.47	62.3 (0.99)	20.23	22.4 (2.45)	34.51	0.80 (0.10)	39.06	35.2 (2.15)	19.37	52.90 (8.39)	50.18
13	46.0 (3.50)	24.06	2.57 (0.24)	29.71	55.4 (2.65)	15.13	26.8 (1.96)	23.15	0.87 (0.10)	36.13	32.7 (2.58)	25.00	47.10 (3.12)	20.95
14	50.0 (2.14)	13.50	3.13 (0.27)	26.94	61.5 (3.31)	17.02	17.3 (2.23)	40.66	0.46 (0.08)	53.31	26.1 (2.19)	26.60	69.21 (6.42)	29.34
15	44.0 (2.09)	15.04	2.67 (0.30)	36.64	59.9 (4.96)	26.21	17.4 (1.26)	22.89	0.52 (0.08)	51.48	28.7 (3.28)	36.07	59.06 (2.99)	16.03
16	51.2 (1.86)	11.49	3.02 (0.12)	12.42	59.1 (1.05)	5.61	17.9 (2.15)	37.95	0.59 (0.07)	42.43	32.1 (2.14)	21.12	68.77 (6.45)	29.66
$LSD_{0.05}$	5.52		0.66		10.74		5.43		0.24		7.63		13.80	

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Table 2. Analysis of variance for yield-related traits and Fusarium-damaged kernels in wheat hybrids

Source	df	Mean square			
		1000-kernel weight	kernel weight per spike	No. of kernels per spike	Fusarium-damaged kernels (%)
Genotype (G)	15	181.40*	1.45*	398.60**	2.39**
Treatment (T)	1	887.12**	499.65**	338.24**	–
Interaction G × T	15	108.72	1.65**	155.02*	–
Error	128	86.34	0.71	70.12	0.59

df – degrees of freedom; *$P < 0.05$; **$P < 0.01$

while in inoculated plants it ranged from 22.89% (hybrid No. 15) to 43.59% (hybrid No. 5). The most variable characteristic was kernel weight per spike, both in control (CV = 12.42–36.64%) and infected (CV = 26.78–68.31%) plants. Coefficients of variation also varied depending on the cross combination; among the examined hybrids the highest coefficients of variation in the control were recorded for hybrid No. 15 (15.04, 36.64 and 26.21% for TKW, KWS and NKS, respectively), whereas in infected plants these traits were the most variable in hybrid No. 5 (43.59, 64.59 and 37.05%, respectively). The FDK percentage was the most variable in hybrid No. 12 (50.18%), and relatively low variation (15.74%) was recorded for hybrid No. 4 (Table 1).

Two-way analysis of variance showed a significant influence of genotype and treatment on all observed characteristics; the genotype x treatment interaction was significant only for the number and weight of kernels per spike (Table 2). The FDK percentage in infected plants was also dependent on genotype (FDK in control plants was not statistically processed because disease symptoms on kernels occurred only sporadically, and in most cases the FDK percentage was equal to zero).

Two homogeneous groups of hybrids were distinguished for individual traits (Table 3). The classification of inoculated plants into groups appeared to be different from that of the control plants. It may be noted that in the case of inoculated plants, three cross combinations, Nos. 2, 12 and 13, were classified in the first group (with higher values) for TKW and KWS and in the second group (with lower values) for FDK. Hybrids No. 2 and 12 also had the highest values of TKW and KWS in the case of the control, whereas No. 13 in the control was classified in the groups with lower values of these traits.

Comparison of the mean values of the observed traits in control and inoculated plants showed that after inoculation all the studied yield-related traits greatly decreased (Table 4). The values of the F statistic for multivariate contrasts for all hybrids indicated their high significance ($P < 0.001$). Figure 1 illustrates that inoculated and non-inoculated hybrids form two entirely separate groups with regard to all traits considered simultaneously. It can also be

Table 3. Homogeneous groups of hybrids for individual traits observed in inoculated and control wheat plants

Trait	Inoculated		Control	
	group mean	hybrids	group mean	hybrids
1000-kernel weight (g)	25.94	12, 13, 2	52.27	9, 14, 4, 5, 16, 7, 8, 2, 12
	17.87	3, 10, 9, 8, 11, 14, 15, 16, 4, 5, 6, 7, 1	45.87	15, 6, 1, 3, 13, 11, 10
Kernel weight per spike (g)	0.775	7, 6, 12, 2, 13	3.24	16, 3, 2, 14, 7, 6, 9, 4, 5, 8, 12, 10
	0.501	11, 4, 14, 8, 9, 15, 3, 10, 5, 1, 16	2.61	1, 13, 15, 11
No. of kernels per spike	34.09	3, 7, 16, 13, 10, 12, 6	67.82	9, 4, 5, 3, 10, 6
	26.24	11, 4, 14, 8, 1, 2, 15, 9, 5	58.81	1, 13, 2, 11, 16, 15, 8, 7, 14, 12
Fusarium-damaged kernels (%)	67.13	4, 10, 9, 16, 14, 3, 8	–	–
	55.42	13, 2, 7, 12, 5, 15, 11, 6, 1	–	–

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Table 4. Estimates of contrasts between control and inoculated wheat plants regarding yield-related traits individually and the *F* statistics for all traits treated simultaneously

Hybrid No.	1000-kernel weight (g)	Kernel weight per spike (g)	No. of kernels per spike	*F* statistics for multivariate contrast[#]
1	23.9**	1.86**	26.8**	32.47**
2	26.8**	2.31**	28.4**	37.62**
3	29.4**	2.56**	35.5**	48.81**
4	32.0**	2.92**	44.2**	64.36**
5	32.0**	2.75**	36.4**	55.58**
6	26.0**	2.48**	30.4**	38.56**
7	31.0**	2.53**	29.4**	48.30**
8	38.6**	2.90**	33.9**	72.42**
9	33.5**	2.76**	35.9**	59.02**
10	32.0**	2.93**	37.9**	57.14**
11	30.3**	2.39**	37.9**	58.75**
12	34.0**	2.67**	27.1**	55.49**
13	19.2**	1.69**	22.7**	20.38**
14	32.7**	2.67**	35.5**	56.95**
15	26.6**	2.15**	31.1**	40.71**
16	33.3**	2.43**	27.0**	52.59**

**$P < 0.01$; [#]for multivariate contrast $F_{0.05} = 2.64$ and $F_{0.01} = 3.85$

observed that the group of inoculated hybrids is more scattered than the control group. The shortest connections between hybrids presented at the dendrite (Figure 2) show that inoculated hybrid No. 13 is positioned closely to hybrids No. 2 and 12, which confirms the classification of hybrids into homogeneous groups.

DISCUSSION

The infection of wheat plants by pathogens of the genus *Fusarium* results in a lowering of yield through a decrease in the number of developed grains and their weight. In the present studies, wheat F_2 hybrids were evaluated in terms of yield-forming traits observed in

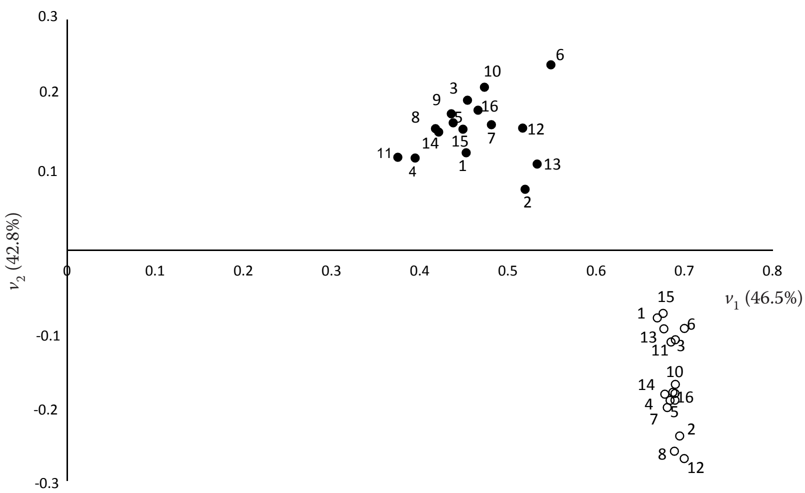

Figure 1. Position of wheat hybrids (1–16) in the coordinates of the first two canonical variables
Unfilled – control; black – inoculated

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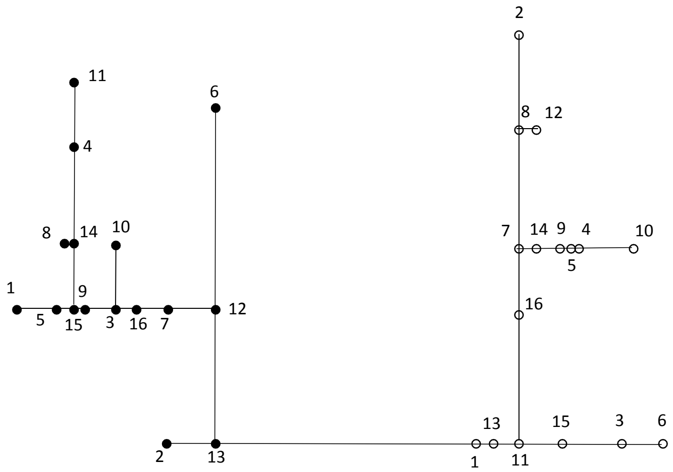

Figure 2. Dendrite of the shortest connections between hybrids (1–16)
Unfilled – control; black – inoculated

plants artificially inoculated with *Fusarium* sp. and in those growing under control conditions. It was found that the variation of hybrids inoculated with *Fusarium* pathogens in terms of yield-forming traits was broader than in those growing under control conditions. This is probably caused by the overlap between two influences, i.e. genetic factors conditioning yield-related traits *per se*, and genetic factors responsible for the reaction to the disease manifested in case of infection (Wiśniewska *et al.* 2016).

The statistical methods used for the evaluation of hybrids made it possible to distinguish the cross combinations which are the most promising for the development of homozygous lines more resistant to infection. In our studies, the results of uni- and multivariate analyses enabled us to select three out of 16 cross combinations (Nos. 2, 12, 13) as especially interesting. These exhibited a low percentage of infected kernels and simultaneously a relatively low reduction of 1000-kernel weight after inoculation. This trait is an important element of yield structure with relatively high heritability and phenotypical stability across environments. As was shown by Varshney *et al.* (2000), this trait, similarly like the resistance to FHB, is quantitatively inherited and controlled by a number of quantitative trait loci (QTL) distributed on almost all of the wheat chromosomes. Wiśniewska *et al.* (2016) found that in wheat some molecular markers (Xgwm46, Xgwm389, Xgwm533) are associated both with the percentage of *Fusarium*-damaged kernels and 1000-kernel weight. This indicates that marker-assisted selection for resistance to FHB can be combined with selection for kernel weight.

The proposed approach to the selection of cross combinations that are promising for the breeding of wheat lines more resistant to the disease may be an alternative to other, more labour-intensive methods, such as diallel or line × tester crossing systems (Griffing 1956; Dobek *et al.* 1989) in which crosses between all chosen genotypes must be made, not only those that are of interest to the breeder. Moreover, using multivariate methods, hybrids combining resistance to the disease with positive values of yield-forming traits can be distinguished. It is important to know at an early stage of the breeding process which combinations should be particularly considered as a potential source for breeding varieties that will be high-yielding and more resistant to Fusarium head blight. A limitation of the proposed approaches is that only the data on one-year experiment are taken into account, but breeders must make a decision on the selection of plant materials for the next steps of breeding on the basis of observations obtained in a given year and they know the risk that such selection may not be very effective. Our work shows that the application of appropriate statistical methods may help to do comprehensive assessment of hybrids at the early stage of breeding.

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