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Original article

Study of Resistance of Common Winter Wheat Lines to the Fusarium Head Blight *(Fusarium Culmorum)*

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Abstract

The study includes 22 lines and varieties of common winter wheat, created in the crop breeding program in IPGR Sadovo and varieties Sadovo and Enola as standards. The trials were conducted during the period 2012-2015 in the infectious field of the Institute. A comparative variety trial was performed in three replicates of the infectious field at the Institute. The sowing was done using the Simson (1969) nesting method (circular sowing). The aim of the study was to investigate the resistance of the breeding materials to the fusarium head blight (Fusarium culmorum). The results showed that there are no immune varieties and lines to the studied phytopathogen. Two common winter wheat lines – PP 787 and BC 7, fall into the group of resistant genotypes. Into the medium sensitive breeding materials group refer the lines DB/A 458, DB 223 and the Fermer variety. The biggest group includes fifteen sensitive genotypes, representing 62.5% of the total number of the study materials. Highly sensitive genotypes to Fusarium culmorum were not reported. The correlation between the fusarium head blight (FHB) and the other economically important diseases (brown rust and powdery mildew) of common winter wheat has been established.

Keywords: Common winter wheat, Resistance, *Fusarium culmorum.*

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INTRODUCTION

The fusarium head blight (FHB) is a serious phytopathological problem for almost all regions of the world in which this crop is grown (Sip et al., 2011). The agents responsible for fusarium head blight in any region depend mainly on the climate and for that reason they may diverse over the years (Van Eeuwijk et al., 1995). The *Fusarium graminearum* predominats in the warmer regions and *Fusarium culmorum* – in the colder areas (Parry et al., 1995). When the wheat flowerings and the weather is rainy, fungus of this types directly infect the spikes and they are causeing large losses as reduction in yield and deterioration of the grain quality (Walter et al., 2010). The warm and wet weather, frequent rainfalls and strong dew during flowering of wheat as well as bipolar maize rotation are the main prerequisites for the development of epiphytes (McMullen et al., 1997). The most effective and economically valuable remedy of combating fusarium head blight is the crop breeding and introduction of resistant varieties into the agricultural production. The first results on variance in the sensitivity of varieties to fusarium head blight (FHB) come from Artur (1891), who puts attention to the urgent need of selection of resistance. Since then, many scientists have investigated the resistance of wheat to the disease and looked for sources of that, but immune varieties and breeding lines have not been found yet (Mladenov, 1987; Dobrev and Kolev, 1998, Karadjova, 2001; Dimov, 2007).

The aim of the study was to investigate the resistance of common winter wheat genotypes of IPGR Sadovo breeding program to fusarium head blight (*Fusarium culmorum*).

Material and Methods

The study included 22 genotypes of common winter wheat created in the breeding program of IPGR, Sadovo and varieties Sadovo1 and Enola as standards (Table 1). The trials were conducted during the period 2012-2015 in the infectious field of the Institute of Plant Genetic Resources – Sadovo, Bulgaria.

A comparative trial by block method in three repetitions on the infectious field of the Institute was conducted. The sowing was done using the Simson (1969) nesting method (circular sowing).

The inoculum preparation of the fusarium head blights agent is performed by the method of Snijders & Van Eeuwijk (1991). For the inoculation of the plants the direct spraying method with spore suspension (8 ml for 10 spikes) in a concentration of 10^6 (1 000 000) macroconidias in 1 ml/water was used. The 10 spikes of genotype were inoculated. To ensure high atmospheric humidity, the inoculated spikes are covered with polyethylene bags for 24 hours.

Under laboratory conditions, all inoculated classes are hand-picked, and the grains are divided into visibly healthy, diseased and shrunken. The following scale is used to report the degree of attack (Mladenov, 1987):

Table 1. Studied common winter wheat genotypes (IPGR Sadovo, Bulgaria)

№	**Line, variety**	**№**	**Line, variety**
1	Sadovo 1	13	DB 380
2	Fermer	14	DB/A 458
3	Ioana	15	DB 313
4	Niky	16	DB 223
5	MX 220/68/1P	17	DB 213
6	MX 178/1	18	DB/A 399
7	MX 187/3	19	BA 607
8	MX 217/69/3,5,7 217/69/3,5,7п	20	BA 471
9	M 181	21	BC 7
10	MX 214/15	22	PP 787
11	MX 215/3	23	DB 295
12	MX 244/1	24	Enola St.

0.0% infected grains – immune lines, varieties; **from 0.01% to 15.00%** infected grains – resistant lines, varieties; **from 15.01% to 25.00%** infected grains – medium sensitive lines, varieties; **from 25.01% to 50.00%** infected grains – sensitive lines, varieties; **over 50.01%** infected grains – strong sensitive lines, varieties.

The percentage of diseased grains and the reduction in yield are calculated as a percentage difference between the grain weight of the control spikes and the grain weight in the inoculated spikes.

Methods of analysis of variance - ANOVA, variation analysis and PC analysis were applied. Data are processed with package programs Statistics 10.

Results and Discussion

The data from the conducted study is presented in Table 2.

With respect to the total number of grains, the coefficient of variation indicates that the lines MX 220/68/1R and M 181 are the least varied on this parameter. The line DB 213 is characterized by the highest coefficient of variation. By the absolute mass of the control, the lowest variance of the attribute was recorded for Fermer variety and the most unstable is the line MX 215/3. Analyzing the results of the infected grains at the indicator percentage by the absolute mass of control, it is evident that it decreases strongly as a result of the pathogen infection. The greatest loss at absolute grain mass relative to the control was reported about the line MX 178/3 - 48.5%. The smallest is the loss at MX 215/3.

The variation analysis showed that for both quantitative signs the variation was low - below 10%. Their variability is almost equal (Table 3).

As a result of the calculated percentage value of the infected grains, the test materials were grouped according to their resistance to the evaluated pathogen (Table 4). The results show that immune breeding lines and varieties of the tested pathogen were not reported. The lack of complete immunity to this phytopathogen is also found in the studies of other authors (Karadjova, 2001; Lin et al., 2004; Dimov, 2007; Chavdarov, 2012).

Two common winter wheat lines – PP 787 and BC 7, fall into the group of resistant genotypes. For PP 787 the lowest percentage of infected grains for the three-year test period (6.8%) was reported.

Four lines and three varieties of common winter wheat are related among the medium sensitive materials. Their infected grains are in the range of 17.3% in the Enola variety to 25.0% on the DB 223 breeding line. The strong variation of the percentage of infected grains in some of the tested materials during the individual years of the study is shown. It is the most indicative for the lines DB/A 458, DB 223 and the Fermer variety. For example, for the Fermer variety, infected grains in the first year were 49.5%, and in the second and third years the infected grains were 5.4% and 19.1%, respectively. These results are confirmation of the study by Dimov (2007), who also established a variation of the resistance in different years.

The biggest is the group of sensitive varieties and breeding lines. It consist fifteen genotypes, representing 62.5% of the total number of studied materials. The lowest percentage of infected grains in this group was reported for the line DB/A 399 (25.9%), and with the most infected grains is the line BA 471 (42.8%). In this group, similar to the previous one, there is a variation of the resistance in different years of the trial.Highly sensitive breeding materials of *Fusarium culmorum* were not reported.

Table 2. Reaction of common winter wheat lines and varieties, inoculated with fusarium head blight (FHB) (*Fusarium culmorum*)

№	Line. variety	Total number of grains			Healthy				Shrunken				Diseased				weight of 1000 grains. the control. g		
		$\bar{x}$	Std. Dev.	CV.%	Grain number	% of the total number	Grain weight. g	% to weight of control	Grain number	% of the total number	Grain weight. g	% to weight of control	Grain number	% of the total number	Grain weight. g	% to weight of control	$\bar{x}$	Std. Dev.	CV.%
1	Sadovo 1	259.3	97.0	37.4	187.0	73.8	8.0	91.4	10.0	3.9	0.2	34.2	62.3	22.5	1.8	57.0	47.5	4.5	9.5
2	Fermer	264.3	85.0	32.2	184.7	71.9	7.9	95.3	9.3	3.4	0.2	45.2	70.3	24.7	2.2	65.1	44.6	2.1	4.6
3	Ioana	320.0	109.8	34.3	185.7	63.1	6.9	84.2	11.3	3.2	0.2	25.9	123.0	33.7	4.4	62.0	46.3	5.0	10.8
4	Niky	259.3	97.4	37.6	174.3	68.7	6.8	89.2	13.5	4.3	0.3	51.1	76.0	28.4	2.4	60.0	45.2	4.6	10.1
5	MX 220/68/1R	253.0	17.3	6.9	140.3	57.0	5.1	80.2	11.3	4.4	0.2	35.3	101.3	38.7	2.7	52.6	44.9	8.8	19.7
6	MX 178/1	343.0	60.8	17.7	206.7	62.3	7.1	95.6	9.3	2.6	0.2	49.3	127.0	35.0	2.4	48.5	35.8	5.7	15.8
7	MX 187/3	283.0	52.5	18.5	200.3	70.5	6.4	91.3	12.7	4.9	0.2	48.5	70.0	24.6	1.6	64.3	35.8	4.7	13.0
8	MX	286.3	90.8	31.7	152.7	57.6	6.0	87.8	16.3	5.4	0.3	37.2	117.3	37.0	3.4	53.2	45.0	4.3	9.7
9	M 181	296.3	33.3	11.2	230.3	78.1	8.1	93.6	7.7	2.7	0.1	44.7	58.3	19.2	1.4	64.1	37.7	4.9	13.1
10	MX 214/15	249.7	60.4	24.2	155.0	60.7	6.2	94.6	11.5	4.4	0.3	49.7	87.0	36.4	2.7	63.5	42.5	4.1	9.6
11	MX 215/3	296.3	62.5	21.1	162.3	58.2	7.0	108.4	8.7	2.9	0.2	43.6	125.3	38.9	3.7	73.6	41.1	8.9	21.5
12	MX 244/1	242.3	73.5	30.3	151.3	64.6	6.1	109.1	5.7	3.1	0.2	48.2	85.3	33.3	2.1	63.5	36.5	4.8	13.2
13	DB 380	262.0	110.1	42.0	153.3	57.5	5.3	90.6	19.5	6.0	0.4	48.0	95.7	38.5	2.2	61.4	38.5	4.1	10.7
14	DB/A 458	288.7	52.8	18.3	216.3	77.3	9.2	94.2	14.3	4.8	0.4	51.1	58.0	17.9	2.0	64.5	45.1	6.1	13.6
15	DB 313	268.7	67.9	25.3	176.3	66.0	7.2	90.3	16.7	5.6	0.4	41.4	75.7	28.4	2.3	66.2	45.9	3.3	7.1
16	DB 223	287.7	91.9	32.0	212.0	72.7	7.8	87.4	6.7	2.3	0.1	35.6	69.0	25.0	1.7	52.0	42.2	3.1	7.2
17	DB 213	291.7	158.9	54.5	166.7	58.6	6.1	84.8	13.0	3.2	0.3	49.6	116.3	39.2	3.5	64.5	43.4	2.8	6.4
18	DB/A 399	276.0	40.0	14.5	189.0	70.1	8.3	93.8	8.0	5.4	0.4	60.4	81.7	28.0	2.6	69.6	46.8	4.9	10.5
19	BA 607	297.3	106.0	35.6	148.0	57.3	6.8	90.6	16.7	4.4	0.5	34.8	132.7	38.3	4.2	56.8	50.1	7.6	15.2
20	BA 471	326.0	91.8	28.1	160.0	50.7	7.3	90.8	21.3	6.4	0.6	48.1	144.7	42.8	4.6	59.7	49.4	5.9	11.9
21	BC 7	242.3	77.6	32.0	208.7	84.4	8.5	95.7	21.5	9.0	0.4	44.3	19.3	9.6	0.5	66.2	42.6	6.1	14.3
22	PP 787	303.3	120.6	39.7	267.0	88.1	11.2	89.1	17.7	5.0	0.3	43.8	18.7	6.8	0.4	50.9	47.5	5.7	12.0
23	DB 295	296.7	105.8	35.7	172.7	62.4	8.3	101.3	15.0	4.2	0.4	57.4	114.0	34.8	3.3	52.2	47.8	4.4	9.2
24	Enola St	288.3	50.6	17.5	230.0	79.9	8.7	92.1	8.3	2.8	0.2	45.2	50.0	17.3	1.4	69.8	41.3	6.0	14.5

Table 3. Variation analysis of the parameters **-** total number of grains and absolute control mass

Parameters	**Number of lines and varieties**	**Mean**	**Min.**	**Max.**	**Std.Dev.**	**CV.%**
Total number of grains	24	282.6	242.3	343.0	26.1	9.2
Weight of 1000 grains. the control	24	43.5	35.8	50.1	4.2	9.7

Table 4. Grouping of breeding materials, according to % of infected grains of *Fusarium culmorum*

I group: Immune lines, varieties are not recorded	
II group: From 0.01% to 15.00% infected grains – resistant lines, varieties	
Line, variety	% infected grains
PP 787	6.8
BC 7	9.6
III group: From 15.01% to 25.00% infected grains – medium sensitive lines, varieties	
Line, variety	% infected grains
Enola St	17.3
DB/A 458	17.9
M 181	19.2
Sadovo 1	22.5
MX 187/3	24.6
Fermer	24.7
DB 223	25.0
IV group: From 25.01% to 50.00% infected grains – sensitive lines, varieties	
Line, variety	% infected grains
DB/A 399	28.0
DB 313	28.4
Niky	28.4
MX 244/1	33.3
Ioana	33.7
DB 295	34.8
MX 178/1	35.0
MX 214/15	36.4
MX 217/69/3.5.7	37.0
BA 607	38.3
DB 380	38.5
MX 220 /68/1P	38.7
MX 215/3	38.9
DB 213	39.2
BA 471	42.8
V group: High sensitive lines. varieties are not recorded	

On Table 5 the results of the dispersion analysis are presented. Obviously, the impact of all evaluated factors has been proven, as well as the interaction between the different factors and all of them together. The influence of factor B (diseases) and the interaction of genotype x diseases (AxB) is the strongest, with almost equal strength – 24.3% and 25.1%, respectively, and the worst impact is shown for the years.

Table 5. Analysis of the variance

Sources of variation	SS	df	MS	F	Sig.	η %
Factor A - Genotype	13463.3	23	585.3	0.68	0.831*	9.1
Factor B - Diseases	35984.1	2	17992.0	3.38	0.118**	24.3
Factor C - Year	8251.7	2	4125.8	0.86	0.487**	5.6
Interaction AxB	37101.3	46	806.5	3.47	0.000***	25.1
Interaction AxC	12705.8	46	276.2	1.19	0.237**	8.6
Interaction BxC	18977.7	4	4744.4	20.44	0.000***	12.8
Interaction AxBxC	21345.3	92	232.0	0.0	0.000***	14.4
Error	0.000					
Total	147829.2					

Principal component analysis (PCA) using the method of the main components in the collection of 24 selected genotypes of common winter wheat based on the main economically important diseases was carried out. As could be seen from the Table 6, about two-thirds of the total variation (75.22%) of the study traits – diseases, are due to the first two major components – PC1 and PC2.

Table 6. Value of the main components

Components	Eigen value	Total variance. %	Cumulative - %
PC 1	1.25	41.71	41.71
PC 2	1.00	33.51	75.22
PC 3	0.74	24.77	100.00

The result from the conducted PCA is presented on Fig. 1 and F2. It provides information of the correlative relationships between the fusarium head blight (FHB) and the brown leaf rust and powdery mildew for the 2012-2015 testing period (Fig.1). Depending on the magnitude of the angle formed between the vectors of each two attributes, correlation dependencies could be determined between them. A positive correlation between the fusarium head blight and the brown leaf rust and a negative relationship between the fusarium head blight (FHB) and the powdery mildew were found.

According to the quadrant, which contains the points of the varieties and the lines and the passing vectors of the signs, it is possible to judge for the corresponding strongest influence of the specific sign (Dragov and Dechev, 2016). In our case, the signs are the studied diseases.

Figure 2 shows the location of varieties and lines in the factorial plane. It is clear that lines 17 and 23 (quadrant 4) have good resistance to *Puccinia recondite* and *Erysiphe graminis*, the vectors of which are located in quadrants 2 and 3. Genotypes 2, 4 and 14 are similar in relation to their immune response to *Fusarium culmorum*. Genotypes 5, 6, 10, 12 and 18 are sensitive to the three economically important diseases. It can be seen that the line 18 is the most sensitive to *Erysiphe graminis*, because the line 18 is located on the vector of this disease. The lines 8, 11 and 13 are sensitive to *Fusarium culmorum*.

The line BC 7 is the furthest from the three disease vectors, which defines it as the most resistant. It is a potential donor of resistance and variation to these pathogens.

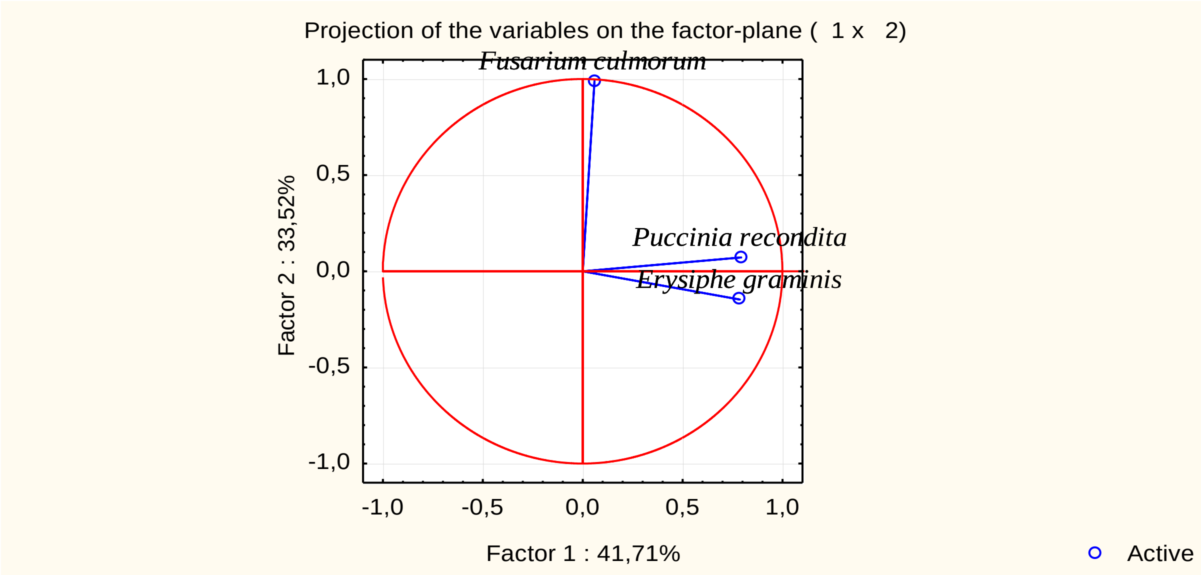

Figure 1. PCA of the fusarium head blight, brown rust and powdery mildew

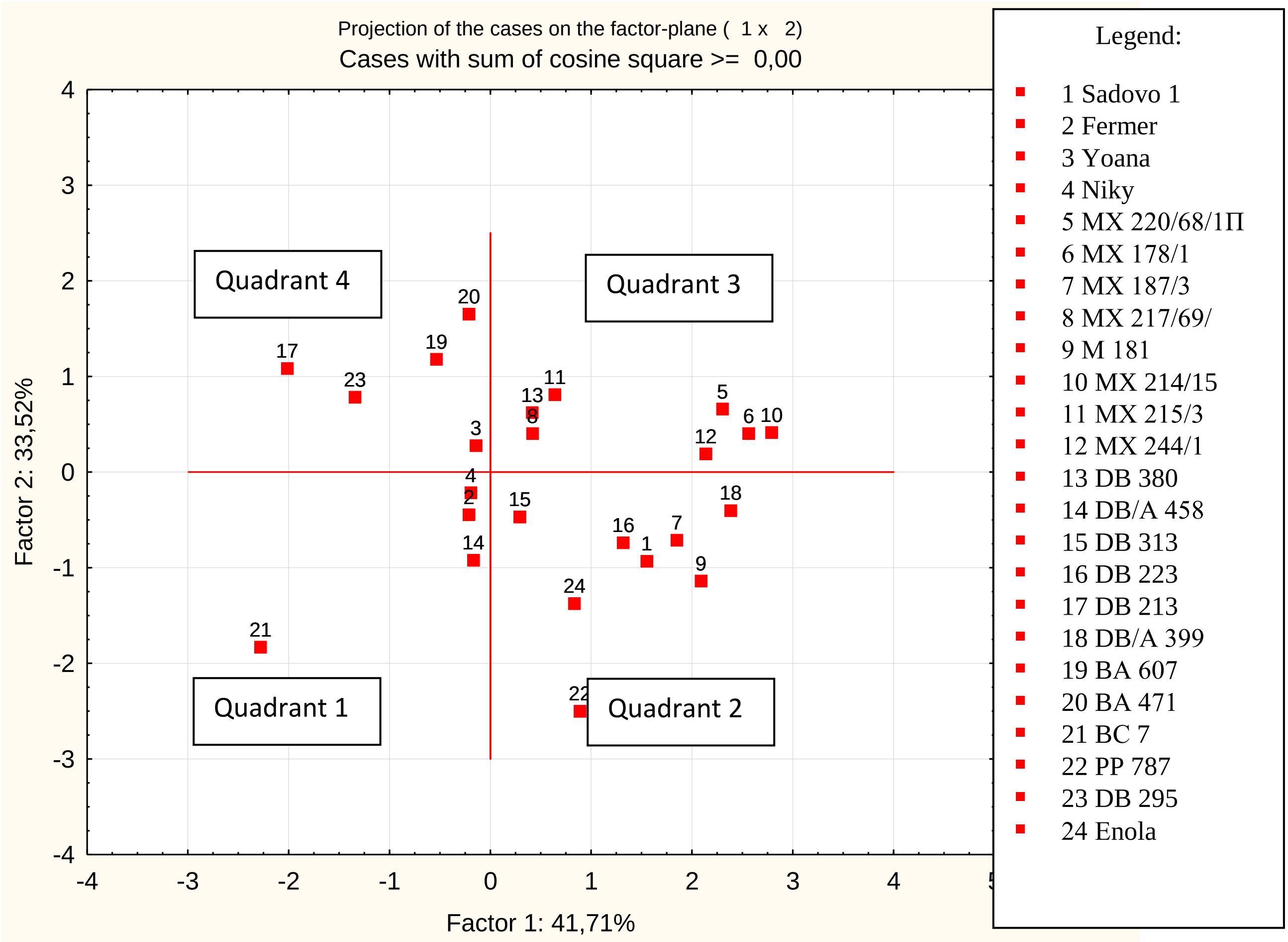

Figure 2. PCA of the lines and varieties

Conclusion

Immune and highly sensitive to fusariosis genotypes are not reported. The two common winter wheat lines – PP 787 and BC 7, are resistant. The biggest group includes fifteen sensitive varieties and breeding lines.

The influence of genotype, years and diseases and their interactions as sources of variation has been proven.

A positive correlation between fusarium head blight and brown leaf rust and a negative relationship between fusarium head blight and powdery mildew were found.

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