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Variation of Fusarium Head Blight Attack in Semidwarf Wheat Cultivars and Relationship with Stripe Rust Attack

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ABSTRACT

Fusarium head blight (FHB) is a major threat to wheat production worldwide. We studied the Type 2 resistance of nineteen winter wheat cultivars under artificial inoculation by injecting spores of two *Fusarium* isolates into central florets of spikes.

Weather conditions and *Fusarium* isolates used for artificial inoculations strongly influenced *Fusarium* attacks, but we also observed significant differences between cultivars. Six semidwarf wheat cultivars carrying *Rht-B1b* allele had significantly lower average Area Under Disease Progress Curve (AUDPC) than other cultivars, including two taller cultivars. Three of the six best resistant wheat cultivars carried the 1A-1R translocation, suggesting a beneficial effect of this rye chromatin presence on reducing the Fusarium head blight attack. Presence of stripe rust attacks significantly increased the area under disease development curve for Fusarium head blight, demonstrating the importance of debilitating effects of other biotic stresses.

Keywords: *Fusarium*, wheat, Area Under Disease Progress Curve (AUDPC), stripe rust, biotic stress, *Rht* gene.

INTRODUCTION

The Fusarium head blight (FHB) of wheat is a major threat to grain production, food safety, and animal health (Spanic et al., 2011; Vaughan et al., 2016). The combination of reduced yield, poor grain quality, and mycotoxin contamination makes FHB a serious threat to the economics of cereal production worldwide (Gilbert and Haber, 2013) as well as to Romania (Suciu et al., 2020; Plăcintă et al., 2022). For example, the direct and indirect economic losses due to FHB outbreaks in the Midwestern United States were estimated at nearly $1 billion per year (Nganje et al., 2004).

Savary et al. (2019) conducted a worldwide survey for yield losses on an individual pathogen and pest basis and estimated a global yield loss of 21.5% for wheat. Yield losses due to FHB ranked second after leaf rust and were particularly high in China, the US Midwest, Canada, South Brazil, Paraguay, Uruguay, and Argentina. FHB not only causes severe losses in both yield and quality but is furthermore of especial relevance for food and feed safety as FHB pathogens contaminate grain with trichothecenes and other toxic fungal metabolites that pose a health risk to human and animals (Buerstmayr et al., 2020).

In Romania, the occurrence rates of *Fusarium* species and chemotypes in common wheat in 2005-2009 were found to be similar to those in Europe. *Fusarium graminearum* 15-ADON isolates predominated (85%) and produced three times less DON than *F. culmorum* 3-ADON isolates; none of the isolates produced NIV (Gagiu et al., 2022).

Plant resistance is the most desirable method for efficiently and reliably controlling FHB. Under epidemic conditions, even the most efficient fungicides may not be good enough to keep the toxin level below the critical threshold particularly on susceptible cultivars, and on the other hand the efficiency of fungicide application to reduce FHB severity and the mycotoxin deoxynivalenol (DON) is higher on moderately resistant cultivars than on susceptible ones (Buerstmayr et al., 2020).

FHB resistance is a very complex trait and, for the five types of resistance reported, it has been associated with more than one

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hundred quantitative trait loci (QTLs) distributed along 20 of the 21 wheat chromosomes (Buerstmayr et al., 2009).

The level of FHB attacks is highly dependent on weather conditions. Additionally, abiotic, and biotic stress factors have the potential to increase the susceptibility to FHB. For example, wheat simultaneously exposed to both aphids and *F. graminearum* exhibits accelerated FHB progression, heightened disease severity, and increased mycotoxin accumulation as compared to plants suffering from *F. graminearum* alone (Drakulic et al., 2015). They showed increased spread and development of host disease, together with greater disease severity and greater accumulation of pathogen DNA and mycotoxin, when aphids were present. Likewise, the severity of FHB was reported to be greater in wheat plants infected with foliar diseases, such as leaf rust (*Puccinia recondita*) and leaf blotch (*Zymoseptoria tritici*) (Mantecón, 2013). Early sprayings caused healthy plants with a lower level of foliar diseases, which improved the physiological state of the crops, producing a higher number of kernels with a lower level of infection caused by the pathogen.

The aim of this work was to identify genetic difference in FHB resistance under artificial inoculation and to establish the effect of stripe rust attack on the response to FHB in several winter wheat cultivars.

MATERIAL AND METHODS

We studied nineteen winter wheat cultivars, tested in the National uniform yield trials in the Southern part of Romania, under artificial inoculations with two *Fusarium* isolates belonging to *Fusarium graminearum* and *Fusarium culmorum* for three years (2021, 2022 and 2023). Seventeen of the studied cultivars (twelve from NARDI Fundulea, two from ARDS Oradea, two from ARDS Caracal and one from ARDS Şimnic) were semidwarf, carrying the *RhtB1b* allele, and only two (Bezostaya 1 a historical check and A4-10 from ARDS Piteşti) carried the allele for normal height.

The weather conditions were different during the study period. During the interval from the inoculation time to the last day of FHB rating the average temperature was the highest (20.5ºC) in 2022 and the highest precipitations with a total of 76.8 mm was recorded in 2021 (Figure 1).

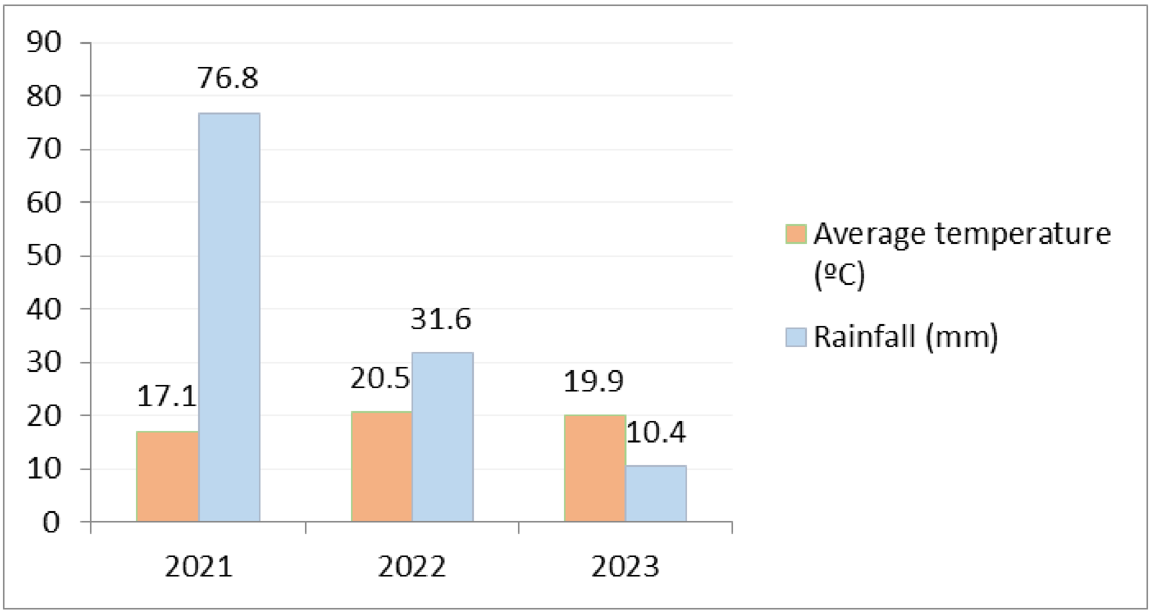

Figure 1. Average temperature and total rainfall during twenty post inoculation days in 2021, 2022 and 2023

We used point inoculations, by injecting *Fusarium culmorum 46* and *Fusarium graminearum 96* spores in central florets of the spike, to estimate the type II resistance, which describes the spread of head blight from the inoculation point to other parts of the head.

Artificial *Fusarium* infections were performed as described by Ittu et al. (2005, 2010) using inoculum obtained from homogenized suspension of *Fusarium* mycelia and conidiospores blended with distilled water. At the time of anthesis, 0.5 ml of inoculum was injected in central florets of five randomly chosen spikes from each plot, per isolate.

FHB ratings of bleached spikelets/entry/ isolate started at 10, respectively, 20 days post inoculation (dpi). The arithmetic mean of the individual successive ratings was used for further calculation of FHB severity (scabbed spikelets, % of control at the onset of symptom development, 20 dpi), and area under disease progress curve (AUDPC) was calculated accordingto the formula:

$$AUDPC = \sum_{i=1}^{n} \{[\frac{Yi+Yi-1}{2}] * (Xi - Xi - 1)\}$$

where:

- Yi is percentage of visibly scabbed spikelets (Yi/100) at the ith observation;

- Xi is day of the ith observation and n is total number of observations.

We assessed stripe rust scores in 2023 by the visual observation method, using a scale from 1 (complete resistance) to 9 (the highest susceptibility).

We analyzed the observed variation of the AUDPC values using a linear mixed model of ANOVA, with Cultivars and Isolates being fixed and Years a random term. To analyze the relationship of FHB attack with the stripe rust attack we used linear regression and Pearson's correlation coefficient.

RESULTS AND DISCUSSION

ANOVA results showed that years, isolates and cultivars contributed most to the observed AUDPC variation. Both cultivars and testing years had significant effects on the variation of AUDPC, when tested against their interaction with years (Table 1). The interaction between cultivars and isolates was not significant when tested against the error, indicating similar response of cultivars to both used isolates.

Table 1. ANOVA for Fusarium head blight AUDPC in 19 winter wheat cultivars artificially inoculated

Source of variation	SS	Df	MS	F	F crit
Cultivars	425503.2	18	23639.07	**4.509606**	1.93
Isolates	678072.5	1	678072.5	**6.444423**	4.11
Cultivars*Isolates	30326.0	18	1684.778	0.947949	1.93
Cultivars*Years	188709.7	36	5241.936	**2.949404**	1.70
Isolates*Years	210437.0	2	105218.5	**59.20175**	3.26
Years	1026624.0	2			
Within	63982.3	36	1777.287		
Total	2623654.0	113			

F values in **bold** are significant at P<5%.

Table 2 illustrates the amplitude of AUDPC variation between the two isolates and between years. Isolate 2 *(F. culmorum)* induced higher FHB symptoms with AUDPC about 50% larger than isolate 1 *(F. graminearum).* In year 2023 AUDPC values were much larger than in both previous years, for both isolates but especially for Isolate 1.

Table 2. AUDPC variation due to *Fusarium* isolates and years

Year	Isolate 1 *(Fusarium graminearum)*	Isolate 2 *(Fusarium culmorum)*
2021	122.4	344.2
2022	138.3	303.8
2023	390.5	465.9
Three years average	217.1	371.3

The AUDPC values averaged over two isolates and three-years were quite different among the tested cultivars, varying from 176.3 to 438.7 (Table 3). Six cultivars had average AUDPC less than 250, not significantly different from each other.

Table 3. AUDPC for Fusarium head blight averaged over two isolates and three years, in 19 winter, wheat cultivars tested under artificial inoculation

Cultivars	2021	2022	2023	Average	Significance groups*
FDL Consecvent	116.0	113.0	300.0	**176.3**	a
Dacic	167.0	213.0	271.0	**217.0**	ab
Caro	145.0	115.0	392.0	**217.3**	ab
Caracal 1	162.0	187.0	337.0	**228.7**	abc
FDL Concurent	220.0	148.0	329.0	**232.3**	abc
FDL Abund	198.0	226.0	309.0	**244.3**	abcd
Biharia	264.0	232.0	369.0	288.3	bcde
A 4-10	250.0	196.0	430.0	292.0	bcde
Amurg	262.0	119.0	509.0	296.7	bcde
Ursita	241.0	202.0	464.0	302.3	cde
Simnic 1619	132.0	281.0	510.0	307.7	cde
Bezostaya 1	243.0	228.0	474.0	315.0	cde
Glosa	254.0	241.0	474.0	323.0	de
Otilia	291.0	284.0	407.0	327.3	de
Pitar	293.0	265.0	460.0	339.3	e
FDL Columna	322.0	211.0	487.0	340.0	e
Voinic	246.0	323.0	463.0	344.0	e
Izvor	293.0	264.0	524.0	360.3	e
FDL Miranda	336.0	353.0	627.0	438.7	f
Average of all cultivars	**233.4**	**221.1**	**428.2**	**294.2**	

*) Cultivars carrying the same letters are not significantly different in their average AUDPC.

It is interesting to note that all more resistant wheat cultivars are semidwarf, *RhtB1b* carriers. Our results confirm that, despite the widely accepted association between higher FHB severity and shorter plant height, this association mostly refers to the type I, not the type II resistance, which was studied in our research (Yan et al., 2011), and at the same time is highly dependent on the genetic background, FHB resistant genotypes retaining a high resistance level even if semi-dwarf alleles are introduced (Buerstmayr and Buerstmayr, 2022).

It is also worth mentioning that the cultivars with best FHB resistance do not have the known Asian sources of resistance in their genealogy and do not carry the *Fhb1*gene. The lowest AUDPC values were observed in newly released cultivars or promising lines having good agronomic values, in contrast with most sources of resistance, which are in backgrounds with poor agronomic and quality traits. We observed that three of the six wheat cultivars with the lowest average AUDPC carry the 1A-1R translocation and this might suggest a beneficial effect of this rye chromatin presence on reducing the FHB attack. Further research is necessary to confirm the potential of this translocation in breeding for FHB resistance.

Trying to understand the higher FHB attack observed in 2023, as compared with both 2021 and 2022, we analyzed the difference between AUDPC determined in 2023 and the average AUDPC for 2021 and 2022. As seen in Table 4, we noticed significant differences between cultivars, which varied from less than one hundred (in cultivars Dacic and FDL Abund) to more than three hundred (in cultivars Simnic 1619 and Amurg).

We tried to analyze the relationship between these differences and several traits of the studied cultivars and found a significant correlation with the notes for the stripe rust recorded, without artificial inoculation, at the same location. It is worth mentioning that the year 2023 was the only year when stripe rust attack was present, causing considerable damage.

The correlation between AUDPC difference between 2023 and 2021-2022 and the notes for stripe rust was 0.62*, meaning that about 38% of the AUDPC variation could be explained by the debilitating effect of the rust on wheat plants. As mentioned earlier, leaf rust was reported to increase the severity of FHB (Mantecón, 2013), but to our knowledge this is the first report mentioning stripe rust as a biotic stress able to aggravate FHB attack.

Table 4. AUDPC difference between years with stripe rust attack (2023) and without stripe rust attack (2021 and 2022)

Cultivars	AUDPC difference between 2023 and 2021-2022	Stripe rust attack (notes*)
Dacic	81	4
FDL Abund	97	2
Otilia	119	2
Biharia	121	1
FDL Concurent	145	7
Caracal 1	162	8
Voinic	178	4
Pitar	181	4
FDL Consecvent	185	1
A 4-10	207	3
FDL Columna	220	7
Glosa	226	6
Bezostaya 1	238	6
Ursita	242	5
Izvor	245	4
Caro	262	6
FDL Miranda	282	7
Simnic 1619	303	8
Amurg	318	8

*) 1 = no attack; 9 = very severe attack.

The figure 2 illustrates the relationship between AUDPC recorded for FHB in 2023 and the notes for stripe rust attack. The regression indicates that on average AUDPC for FHB increased by 18 points for each unit of stripe rust attack. Large deviations from the regression lines are also visible, suggesting that cultivars Dacic, FDL Concurent showed lower FHB symptoms than expected based on their stripe rust attack.

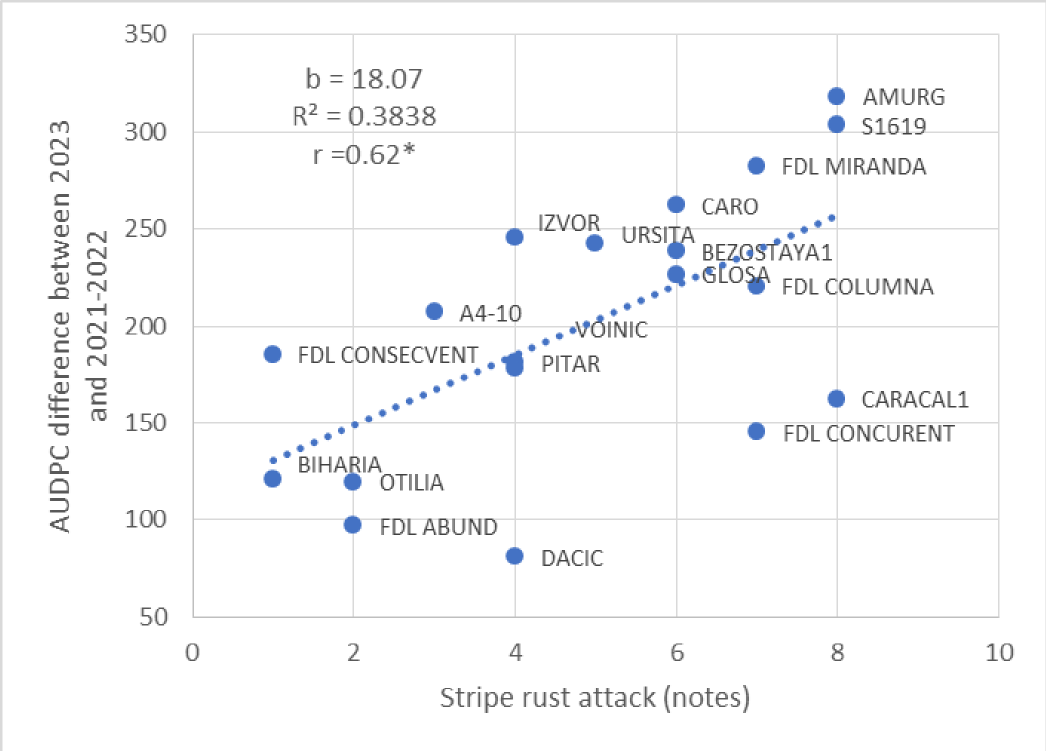

Figure 2. Relationship between the attack of stripe rust and the difference of AUDPC for Fusarium head blight in 2023 (with heavy stripe rust attack) and in the average of 2021-2022 (without stripe rust)

CONCLUSIONS

Weather conditions and *Fusarium* isolates used for artificial inoculations strongly influenced Fusarium head blight attacks, but we also observed significant differences between cultivars.

Six semidwarf wheat cultivars carrying *Rht-B1b* allele had significantly lower average Area Under Disease Progress Curve than other cultivars, which included two taller cultivars.

Three of the six best resistant wheat cultivars carried the 1A-1R translocation, suggesting a beneficial effect of this rye chromatin presence on reducing the *Fusarium* attack.

Presence of stripe rust attacks significantly increased the area under disease development curve for Fusarium head blight, demonstrating the importance of debilitating effects of other biotic stresses.

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