

GENETIC VARIABILITY AND HERITABILITY OF WHEAT (*TRITICUM AESTIVUM* L.) RESISTANCE IN *FUSARIUM AVENACEUM* (FR.) SACC.

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The results of the research on the reaction of 45 common wheat genotypes to the treatment of grains with the culture filtrates of 3 *Fusarium avenaceum* isolates are presented. By the cluster analysis (distribution dendrograms, k-mean method) was found: the degree of similarity of genotypes based on different growth and development traits; differentiated phenotypic plasticity of the growth organs in response to the action of the pathogen. Genotypes with decreased sensitivity to the action of isolates have been identified. It has been established that in the interaction of wheat with *F. avenaceum* the variability of the characters has a strong genetic determinism, and the association of the high level of the coefficient of heritability in the broad sense with the advanced genetic progress shows that in the control of growth and development characters are involved which offers real chances of success in improving the resistance of wheat to *F. avenaceum*.

Keywords: wheat, genotype, *Fusarium avenaceum*, variability, heritability, resistance.

INTRODUCTION

According to our recent data, in the fungal complex that produces root rot in common winter wheat (*Triticum aestivum* L.) in the conditions of the Republic of Moldova, has grown considerably the incidence of *Fusarium avenaceum* (Fr.) Sacc. which reached the rate of 11.4% (Lupascu, Gavzer, 2021).

The fungus *F. avenaceum* was first described in 1886 and is one of the most widespread plant pathogenic species, frequently involved in the rot of root, stem, fruit, plant decay (Peters *et al.*, 2008; Sørensen *et al.*, 2009; Sakoda *et al.*, 2011), causing huge economic losses to agricultural crops. For example, under Slovenian conditions, the causative agent of wet apple rot has been found to be *F. avenaceum* (Sorensen *et al.*, 2009). The involvement of this fungus in the severe development of soybean root rot has been established (Chang *et al.*, 2018); onion (Zhang *et al.*, 2016), *Rubus idaeus* fruit (Wang *et al.*, 2016), corn panicle (Ma *et al.*, 2019), wheat and barley grown in Europe and Asia (Ponts *et al.*, 2020).

Along with direct economic losses, for cereal growers, especially wheat and barley, the fungus is also a food safety concern because it is also an active producer

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of mycotoxins in grains such as moniliformin, eniatin, bovericin (Jestoi *et al.*, 2004; Yli-Mattila *et al.*, 2006; Uhlig, Jestoi, Parikka, 2007; Ponts *et al.*, 2020).

The analysis of the ISSR (Inter Simple Sequence Repeats) DNA sequences for the beta-tubulin DNA sequences of 26 monospore strains of *F. avenaceum*, isolated from grass plants, found a great genetic diversity within the species *F. avenaceum* (Yli-Mattila *et al.*, 2018)

DNA analysis (RAPD) -PCR also showed considerable genetic variation between *F. avenaceum* isolates. Genetic diversity was also confirmed by the high degree of vegetative incompatibility between 20 isolates. However, no relationships were found between pathogenicity, RFLP group, RAPD group and vegetative compatibility group (Satyaprasad, Bateman, Ward, 2000). According to some opinions, variations in *F. avenaceum* evolved (i) through single nucleotide polymorphisms (SNPs), (ii) through DNA length variations caused by insertion / deletion sequences (Indels) (Ponts *et al.*, 2020).

Incorporating genetic resistance into crop plants is considered the most effective and sustainable method of counteracting root rot, although resistance is often of a quantitative nature. For strategies to improve disease resistance, it is necessary to know the genetic basis of the reaction to it (Williamson-Benavides, Dhingra, 2021).

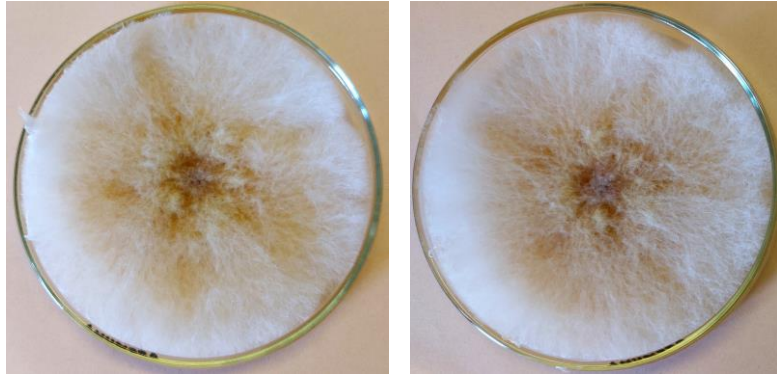
Although it is widely acknowledged that the use of resistant wheat varieties remains the most effective and economical way to control root rot, there are currently insufficient resources for disease-resistant germplasm and there are few studies on the identification of genetic sites that control resistance at root rot (Gupta *et al.*, 2018).

According to the author Snijders (2004), the resistance of wheat to *Fusarium* species is complex and includes several components – resistance to fungal penetration, resistance to colonization, mechanisms that influence the content of toxins (DON – deoxynivalenol) in grains. Research aimed at identifying and accumulating as many resistance QTLs in elite lines as possible is considered promising.

Based on the above, the aim of our research was to elucidate the variability of phenotypic plasticity of common wheat genotypes in winter in response to the action of the fungus *F. avenaceum* and the peculiarities of heritability of wheat resistance to pathogen.

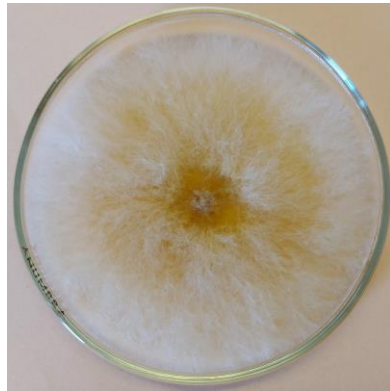
MATERIAL AND METHODS

In our research, the fungi have been isolated on the PDA medium from wheat plants with signs of root rot at the stem base (Tuite, 1969). The identification of pathogens was performed by the macro- and microscopic characters according to the mycological key (Barnett, Hunter, 1998) (Figs. 1, 2).



Isolate 1

Isolate 2



Isolate 3

Fig. 1. *F. avenaceum* colonies.Fig. 2. *F. avenaceum* conidia (300x).

45 winter wheat genotypes – representing varieties and lines of different ecological and geographical origin – were tested. The seeds were obtained from genotypes grown on the IGFPP experimental sector.

For laboratory testing were used the culture filtrates (CF) of the *F. avenaceum* fungus prepared by inoculating the mycelium into the Czapek-Dox liquid medium containing the following components (g / l of distilled water): NaNO₃ – 3.0; K₂HPO₄ – 1.0; MgSO₄ · 7H₂O – 0.5; KCl – 0.5; FeSO₄ · 7H₂O – 0.01; sucrose – 30.0 (Tuite, 1969). Wheat kernels were treated with CF for 18 hours. As a control served the grains kept in the distilled water. Cultivation of the seedling took place in Petri dishes on filter paper wetted with distilled water, at a temperature of 18–19°C for 6 days. As test indices of plant reaction served the important growth and developing characters at early stage of ontogenesis – germination, root length and stem length.

A considerable contribution to genetic diversity research of the plants has the classification method that would objectively identify the degree of similarity or difference between genotypes. For this purpose, two methods of cluster analysis were used: the agglomerative-iterative method of constructing distribution dendrograms and the centroid method of *k*-means, both methods being used successfully in genetic and breeding research (Ravishanker *et al.*, 2013; Koija, Sabaa, 2015; Nandini *et al.*, 2017).

In order to perform the *k*-means clusterian analysis we have programmed the distribution of the set of genotypes in 3 clusters, according to the possible values – large, medium and small of the characters (germination, root length, stem length, seedling length, vigor index) that served as cases.

The data were statistically processed in the STATISTICA 8 software package.

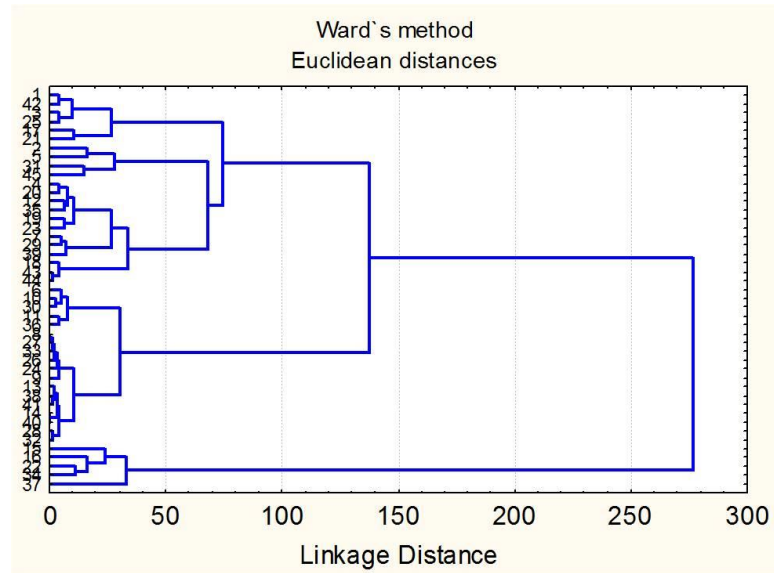
RESULTS AND DISCUSSION

In order to establish the similarity / differences between the wheat genotypes studied based on the reaction to the 3 strains of *F. avenaceum*, the dendrograms of the distribution of genotypes according to some important biological indices were researched. Thus, it was found that the distribution of genotypes in clusters according to germination, root length, stem length, seedling length, vigor index was different, which indicates that the similarity of wheat genotypes was determined by the specificity of phenotypic plasticity of growth organs in response to *F. avenaceum* isolates (Fig. 3).

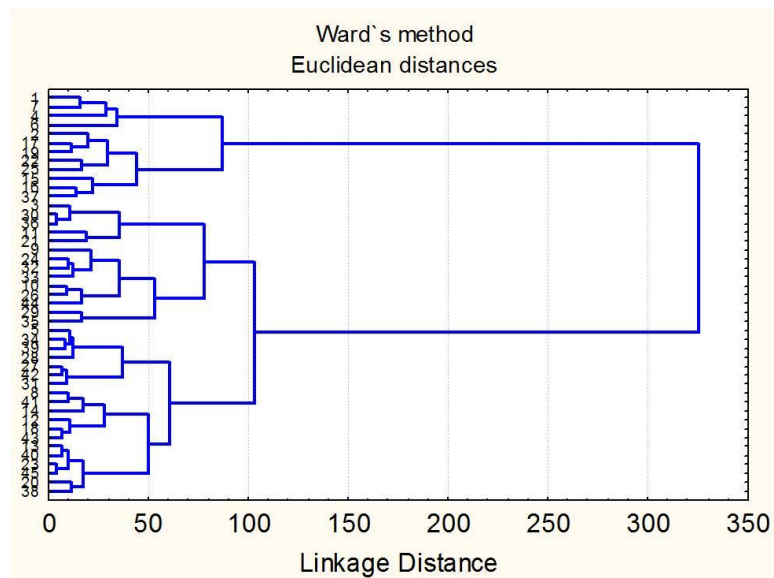
For more accurate quantification of clusters of wheat genotypes and the role of *F. avenaceum* fungus isolates in their differentiation, cluster analysis was applied according to the *k*-means method.

The analysis of the cluster variance in which 4 variants served as cases – control, CF1, CF2, CF3 showed that the intercluster variance was, with one exception

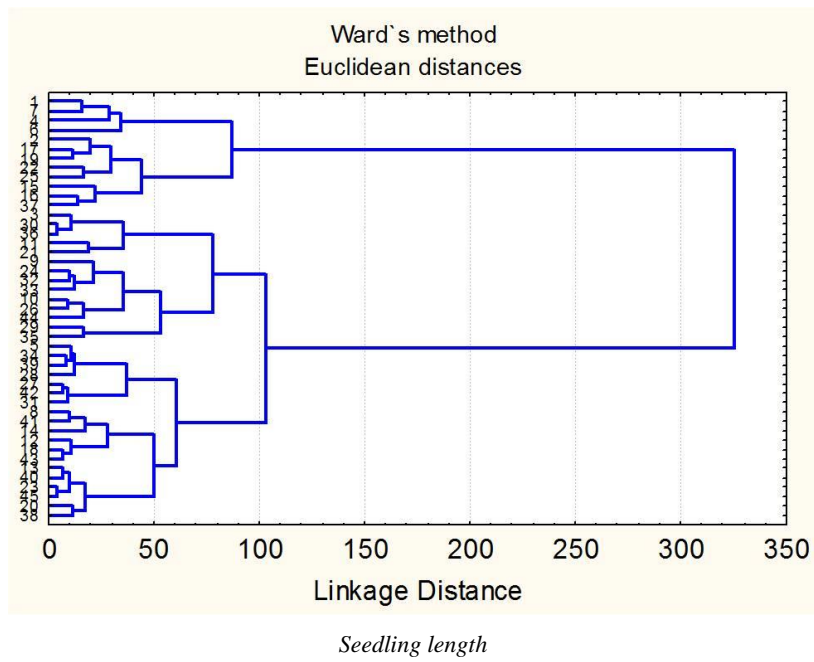
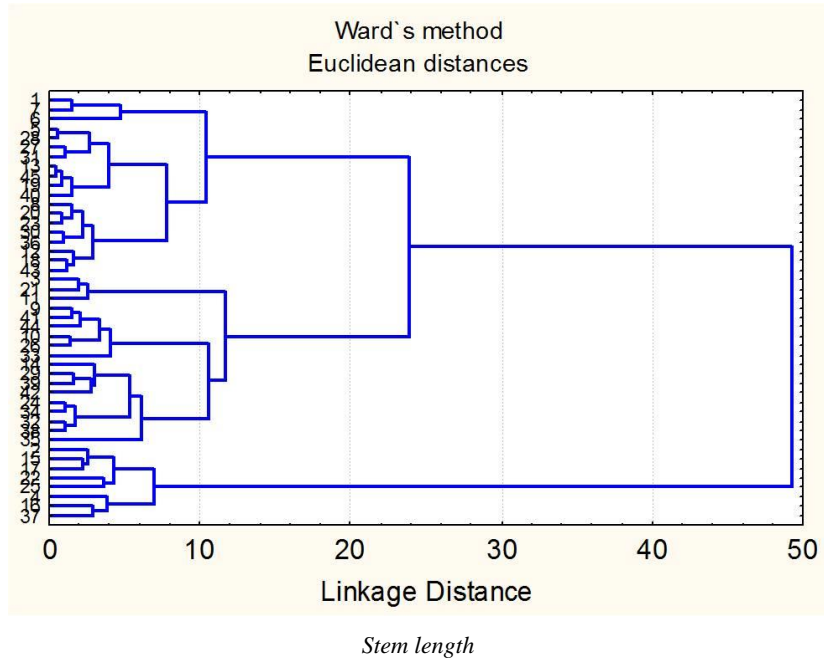
(CF3, root length) much larger than the intracluster variance, which denotes the classification success of genotypes in distinct clusters based on all characters studied (Tab. 1).

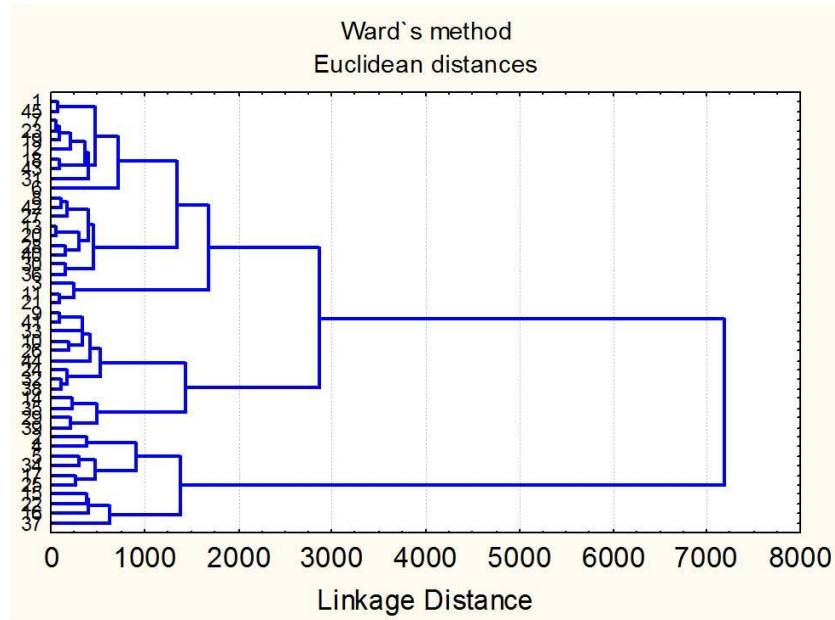


Germination



Root length





Vigor index

Fig. 3. Distribution dendrogram of common wheat collection genotypes based on the reaction of growth and development characters to *F. avenaceum* strains.

Table 1

Analysis of the clusters variance of wheat genotypes

Trait	Variant	Variance between clusters	df	Variance within clusters	df	F	p
Germination	Control	858.656	3	265.533	41	44.194	0,000
	CF1 <i>F.avenaceum</i>	5931.513	3	938.343	41	86.391	0,000
	CF2 <i>F.avenaceum</i>	2935.827	3	957.846	41	41.889	0,000
	CF3 <i>F.avenaceum</i>	2213.153	3	1573.990	41	19.216	0,000
Root length	Control	6115.552	3	1673.486	41	49.943	0,000
	CF1 <i>F.avenaceum</i>	3475.694	3	2917.591	41	16.281	0,000
	CF2 <i>F.avenaceum</i>	3533.946	3	2815.078	41	17.157	0,000
	CF3 <i>F.avenaceum</i>	2862.525	3	3274.317	41	11.948	0,000

Table 1 (continued)

Trait	Variant	Variance between clusters	df	Variance within clusters	df	F	p
Stem length	Control	2210.361	3	458.072	41	65.947	0,000
	CF1 <i>F.avenaceum</i>	1566.465	3	771.989	41	27.731	0,000
	CF2 <i>F.avenaceum</i>	1467.030	3	737.500	41	27.186	0,000
	CF3 <i>F.avenaceum</i>	1693.999	3	927.655	41	24.957	0,000
Seedling length	Control	139.375	3	43.138	41	44.156	0,000
	CF1 <i>F.avenaceum</i>	102.901	3	41.122	41	34.199	0,000
	CF2 <i>F.avenaceum</i>	69.783	3	58.906	41	16.190	0,000
	CF3 <i>F.avenaceum</i>	86.280	3	74.322	41	15.866	0,000
Vigor index	Control	1550070	3	687273	41	30.824	0,000
	CF1 <i>F.avenaceum</i>	2626518	3	923474	41	38.870	0,000
	CF2 <i>F.avenaceum</i>	1101859	3	1094309	41	13.761	0,000
	CF3 <i>F.avenaceum</i>	1828041	3	1075156	41	23.237	0,000

Of great importance for obtaining uniform sowings with vigorous plants is the vigor index which is strongly influenced by environmental conditions, including Fusarium pathogens (Hassani, Zare, Khaledi, 2019). As it is known, the determinants of the vigor index are the germination and the length of the seedlings. The classification of genotypes according to germination in the control variants, CF1, CF2, CF3 showed that the most efficient was cluster 4, consisting of 22 genotypes that recorded an average germination of 99.3% in the control variant and 97.08–97.58% in the variants with cultures filtrates. Cluster 1 (n = 5) presented the lowest indicators: 85.1% – in the control variant and 61.3–77.3% – in the CF variants. The mean level of clusters 2 (n = 6) and 3 (n = 12) was slightly decreased compared to cluster 4 (Tab. 2).

Table 2

Descriptive statistics of wheat genotype clusters formed based on wheat kernels germination

Variant	Mean, %	Standard deviation	Members of cluster
Cluster 1, n = 5			
Control	85.11	6.79	15 – Urbanus, 16 – Python, 22 – Neven, 34 – Judita, 37 – Bucovina SV.
CF1 <i>F.avenaceum</i>	61.33	5.27	
CF2 <i>F.avenaceum</i>	77.33	4.63	
CF3 <i>F.avenaceum</i>	73.77	12.39	

Table 2 (continued)

Variant	Mean, %	Standard deviation	Members of cluster
Cluster 2, n = 6			
Control	99.45	0.93	1 – Avant, 3 – Moldova 30, 17 – Zvitlaga, 21 – Aneta, 25 – Nasnaga, 42 – Moldova 66.
CF1 <i>F.avenaceum</i>	80.74	7.53	
CF2 <i>F.avenaceum</i>	95.55	3.52	
CF3 <i>F.avenaceum</i>	92.96	2.29	
Cluster 3, n = 12			
Control	97.97	2.21	2 – S.i. Cubani 101, 4 – Selania, 5 – Alunis, 12 – Select, 18 – Apullum, 19 – Turda 81, 23 – Vyara, 31 – ITC-30, 35 – Avenue, 43 – Kuialnic, 44 – Miranda, 45 – Centurion.
CF1 <i>F.avenaceum</i>	93.24	5.64	
CF2 <i>F.avenaceum</i>	82.22	8.08	
CF3 <i>F.avenaceum</i>	92.88	7.25	
Cluster 4, n = 22			
Control	99.25	1.05	6 – Capriana, 7 – BȚ 16-04, 8 – Accent, 9 – Balada, 10 – Niconia, 11 – BT 43-42, 13 – Trublion, 14 – Numitor, 20 – Ardeal, 24 – Messino, 26 – L 641/19, 27 – L 642/19, 28 – L 643/19, 29 – Cuibo, 30 – ITC 12, 32 – GK Koros, 33 – Tika-Taka, 36 – Rotax, 38 – Speranta, 39 – Dacia, 40 – Transilvania, 41 – Amor.
CF1 <i>F.avenaceum</i>	97.33	3.05	
CF2 <i>F.avenaceum</i>	97.58	2.10	
CF3 <i>F.avenaceum</i>	97.08	4.12	

The classification of genotypes according to seedling length showed that the most advanced is cluster 3 (n = 12), the average of which was 17.01 cm in the control variant and 14.4–15.5 cm – in the CF variants (Tab. 3).

Regarding the vigor index, it was found that cluster 3 consisting of 13 genotypes recorded except CF3, the highest values, compared to other clusters. It is worth mentioning that in this cluster were located practically the same genotypes as in the most efficient cluster obtained according to the length of the seedling – cluster 3. It follows that in our research this parameter determined the vigor index to a greater extent than the germination factor (Tab. 4).

Table 3

Descriptive statistics of wheat genotype clusters formed based on seedling length

Variant	Mean, %	Standard deviation	Members of cluster
Cluster 1, n = 16			
Control	14.31	0.92	1 – Avant, 5 – Alunis, 6 – Capriana, 7 – BȚ 16-04, 8 – Accent, 12 – Select, 13 – Trublion, 19 – Turda 81, 20 – Ardeal, 23 – Vyara, 27 – L 642/19, 28 – L 643/19, 31 – ITC 30, 40 – Transilvania, 43 – Kuialnic, 45 – Centurion.
CF1 <i>F.avenaceum</i>	13.86	0.75	
CF2 <i>F.avenaceum</i>	12.89	1.09	
CF3 <i>F.avenaceum</i>	13.94	1.32	

Table 3 (continued)

Variant	Mean, %	Standard deviation	Members of cluster
Cluster 2, n = 9			
Control	15.78	0.60	3 – Moldova 30, 10 – Niconia, 11 – BT 43-02, 18 – Apullum, 21 – Aneta, 24 – Messino, 26 – L 641/19, 30 – ITC 12, 36 – Rotax.
CF1 <i>F.avenaceum</i>	13.10	1.45	
CF2 <i>F.avenaceum</i>	14.78	0.77	
CF3 <i>F.avenaceum</i>	15.29	1.06	
Cluster 3, n = 12			
Control	17.01	1.28	9 – Balada, 14 – Numitor, 29 – Cuibo, 32 – GK Koros, 33 – Tika-Taka, 34 – Judita, 35 – Avenue, 38 – Speranta, 39 – Dacia, 41 – Amor, 42 – Moldova 66, 44 – Miranda.
CF1 <i>F.avenaceum</i>	15.47	0.72	
CF2 <i>F.avenaceum</i>	14.73	1.12	
CF3 <i>F.avenaceum</i>	14.40	1.61	
Cluster 4, n = 8			
Control	11.87	1.18	2 – S.i. Cubani 101, 4 – Selania, 15 – Urbanus, 16 – Python, 17 – Zvitlaga, 22 – Neven, 25 – Nasnaga, 37 – Bucovina, SV.
CF1 <i>F.avenaceum</i>	10.92	1.21	
CF2 <i>F.avenaceum</i>	11.54	1.80	
CF3 <i>F.avenaceum</i>	11.02	1.24	

Table 4

Descriptive statistics of wheat genotype clusters formed on the basis of the vigor index

Variant	Mean, %	Standard deviation	Members of cluster
Cluster 1, n = 8			
Control	1107.59	155.65	2 – S.i. Cubani 101, 4 – Selania, 15 – Urbanus, 16 – Python, 17 – Zvitlaga, 22 – Neven, 34 – Judita, 37 – Bucovina, SV.
CF1 <i>F.avenaceum</i>	803.28	226.82	
CF2 <i>F.avenaceum</i>	942.71	179.49	
CF3 <i>F.avenaceum</i>	911.89	212.21	
Cluster 2, n = 19			
Control	1444.08	77.51	1 – Avant, 3 – Moldova 30, 5 – Alunis, 7 – BT 16-04, 11 – BT 43-42, 12 – Select, 13 – Trublion, 19 – Turda 81, 20 – Ardeal, 21 – Aneta, 23 – Vyara, 25 – Nasnaga, 28 – L 643/19, 30 – ITC 12, 31 – ITC 30, 36 – Rotax, 40 – Transilvania, 43 – Kuialnic, 45 – Centurion.
CF1 <i>F.avenaceum</i>	1200.09	161.10	
CF2 <i>F.avenaceum</i>	1248.31	183.91	
CF3 <i>F.avenaceum</i>	1320.50	136.86	
Cluster 3, n = 13			
Control	1663.98	144.53	8 – Accent, 9 – Balada, 14 – Numitor, 24 – Messino, 27 – L 642/19, 29 – Cuibo, 32 – GK Koros, 33 – Tika-Taka, 35 – Avenue, 38 – Speranta, 39 – Dacia, 41 – Amor, 42 – Moldova 66.
CF1 <i>F.avenaceum</i>	1501.63	76.49	
CF2 <i>F.avenaceum</i>	1413.57	104.82	
CF3 <i>F.avenaceum</i>	1371.13	178.58	

Table 4 (continued)

Variant	Mean, %	Standard deviation	Members of cluster
Cluster 4, n = 5			
Control	1508.93	199.30	6 – Capriana, 10 – Niconia, 18 – Apullum, 26 – L 641/19, 44 – Miranda.
CF1 <i>F.avenaceum</i>	1430.16	80.60	
CF2 <i>F.avenaceum</i>	1220.44	178.97	
CF3 <i>F.avenaceum</i>	1631.55	100.12	

As is well known, factor analysis is a multidimensional procedure that highlights the role of different independent factors in the variability of a dependent character (Sharifi, Astereki, Pouresmael, 2018).

The analysis of the influence of the *genotype x F. avenaceum isolates* relationship on the growth and development characteristics of wheat plants found that in most cases the isolate factor showed a more important source of variation than the genotype or the *genotype x isolate* interaction factor and varied within the limits of 60.7–68.6%. Only in the case of the strain, the share of wheat genotype (46.7%) slightly exceeded that of isolate (44.8%) (Tab. 5).

Table 5

Factorial analysis of the influence of *wheat x F. avenaceum* interactions on wheat seedling growth

Source of variation	DF	Germination		Root length		Stem length		Seedling length		Vigor index	
		ASS	SSV, %	ASS	SSV, %	ASS	SSV, %	ASS	SSV, %	ASS	SSV, %
Genotype	44	715*	30.2	1087*	25.5	504*	46.7	26,8*	30.1	512440*	25.3
<i>F. av.</i> isolate	3	1435*	60.7	2797*	65.6	484*	44.8	54,1*	60.7	1390651*	68.6
<i>Genotype x F. av.</i> isolate	132	118*	5.00	244*	5.7	55*	5.1	5,1*	5.7	77101*	3.8
Error	360	98	4.1	138	3.2	37	3.4	3,1	3.5	46702	2.3

*- $p < 0,05$; DF – Degrees of freedom; ASS – Average Sum of Squares; SSV – Share in Source of Variation

The study of heritability, variability and genetic progress is of great importance for elucidating the chances of success of using one or another character in the breeding process in order to create a new variety (Bello *et al.*, 2012; Taneva *et al.*, 2019).

The data obtained show that the GCV (%) which is the best index for assessing the genetic variance recorded an average level for the characters of germination and root length – 15.46% and 19.25%, respectively, and for the stem length, seedling length, vigor index – a high level: 26.36; 20.13; 30.12%, respectively (Tab. 6).

Table 6

Heritability and variability of common wheat growth traits under the influence of *F. avenaceum* isolates

Parameter	Germination	Root length	Stem length	Seedling length	Vigor index
σ^2_G	205.67	316.33	155.67	7.9	155246
σ^2_P	303.67	454.33	192.67	11.0	201948
GCV, %	15.46	19.25	26.36	20.13	30,12
PCV, %	18.78	23.07	29.33	23.76	34,35
h^2 , %	67.73	69.62	80.80	71,82	76,87
GAM, %	19.16	24.99	31.58	25.50	38,28

Heritability is a measure of the phenotypic variance attributed to genetic factors and has a predictive function in plant breeding. According to our data, the coefficient of heritability in the broad sense (h^2) varied within the limits of 67.7–80.8% for the characters studied, which indicates that the genetic variance identified by the GCV parameter (%) is inherited at a high level (Tab. 6).

Knowing the level of heritability is useful for predicting expected progress, but heritability itself does not provide information on the extent of genetic improvement that results from individual genotype selection. Therefore, knowledge about heritability associated with genetic advance is more valuable (Rosmaina *et al.*, 2016).

Thus, the genetic advance (GAM,%) registered an average level (19.16%) for germination, but a high level – for the other characters (24.99–38.28%), which indicates that being associated with a high level of heritability ensures a good relevance of the studied characters in order to create wheat genotypes resistant to *F. avenaceum* attack.

CONCLUSIONS

1. The cluster analysis (distribution dendrograms) of 45 common wheat genotypes of autumn wheat, of different ecological-geographical origin, treated with *F. avenaceum* culture filtrates showed that their similarity depends a lot on the tested organ, which indicates that in the reaction of wheat to the pathogen manifests a specific phenotypic plasticity of the organ of growth and development.

2. A cluster of 13 wheat genotypes was identified by the *k*-mean method of cluster analysis – Accent, Balada, Numitor, Messino, L 642/19, Cuibo, GK Koros, Tika-Taka, Avenue, Speranta, Dacia, Amor, Moldova 66 with high vigor index of seedlings at the action of culture filtrates of *F. avenaceum* 3 isolates.

3. It was found that the share of the *F. avenaceum* isolate factor in the source of variation of germination, embryonic root length, stem length, seedling length, vigor index varied within the limits of 44.8–68.6%, and wheat genotype – 25.3–46.7%.

4. For the growth and development characters studied, at the interaction of wheat with *F. avenaceum* culture filtrates, the genotypic coefficient of variation varied within the limits of 15.46–30.12%, the coefficient of heritability in the broad sense – 67.7–80.8%, and genetic progress – 19.16–38.28%. The data obtained show that in the interaction of wheat with *F. avenaceum* the variability of the characters has a strong genetic determinism, and the association of the high level of the coefficient of heritability in a broad sense with the advanced genetic progress shows that in the control of growth and development characters are involved, which offers a real chance of success in improving the resistance of common wheat to *F. avenaceum* fungus.

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