THE ROLE OF WHEAT GENETIC VARIABILITY IN THE INTERACTION WITH *DRESCHLERA SOROKINIANA*

GALINA LUPASCU[1]*, SVETLANA GAVZER[1], NICOLAE CRISTEA[1], ELENA SAȘCO[1]

Abstract: The treatment of the grains of 10 genotypes of common wheat with 3 filtrates of *Drechlera sorokiniana* culture, demonstrated the manifestation of specific reactions of the seedling, depending on the genotype, growth organ, fungus isolate. By cluster analysis (dendrograms, *k*-means methods) the resistant genotypes to the pathogen were identified. The factorial analysis demonstrated that in the reaction of the growth organs to *D. sorokiniana* isolates, the weight of the genotype factor was 38.2 ... 45%, of the isolate – 34.1 ... 38.8%. For the vigor index, a higher influence of the isolate was recorded – 42.6%, with the genotype factor returning 37.5%. The share of *genotype x isolated* interactions varied depending on the analyzed character of the plant within the limits of 12.3–19.3% ($p<0.5$). The pronounced association of fairly high values of the heritability coefficient and the genetic advantage for the growth organs reveal the additive control of resistance, which offers chances of success in the creation of genotypes resistant to the pathogen.

Keywords: wheat, *Drechlera sorokiniana*, variability, heritability, genetic advantage.

INTRODUCTION

Wheat is among the top ten and most cultivated crops in the world. Several diseases cause losses in wheat production in different parts of the world. The hemibiotrophic fungus *Drechslera sorokiniana* (Sacc.) Subram. and Jain., syn. *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is one of the most devastating disease agents of wheat and other grassy cereal crops worldwide. The pathogen can attack the grains, roots, stems, leaves, diminishes the vegetative mass of the plant, reduces the quality of the grains and is capable of producing toxins, such as sorokininin and helmintosporol. Seed infection with *B. sorokiniana* can cause black embryo disease, reducing seed quality and germination, and is considered a primary source of inoculum for diseases such as common root rot and gray leaf spot (helmintosporiosis). According to some opinions, no wheat variety is immune to this pathogen (Al-Sadi, 2021; Roy *et al*., 2023; Tunali *et. al*., 2023).

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The species *B. sorokiniana* has been found to represent a continuous line of isolates that vary in virulence and aggressiveness with specific and non-specific interactions (Duveiller, Altamirano, 2000), which makes them particularly suitable for screening plant resistance to helminthosporiosis (Arabi, Jawhar, 2012).

Commercial cultivars are moderately resistant or susceptible, and such cultivars could be heavily infected in an environment conducive to disease by various foliar and root diseases caused by the fungus. A number of fungicides, especially those of the triazole group, have shown good effects in reducing the disease. Along with agrotechnical measures, such as rotation, sowing time, balanced fertilization, application of fungicides (Roy *et al*., 2023; Tunali *et. al*., 2023), the cultivation of resistant varieties is the most effective means of combating diseases.

According to the authors (Sharma, Dubin, 1996), the increased resistance of wheat due to the cultivation of multiline populations led to the reduction of the disease by up to 57%, and the increase in yield – by up to 8.6% compared to the component lines. The evaluation of wheat germplasm under different agroclimatic conditions contributed to the identification of resistant genotypes (SW 89-5193, SW 89-3060, SW 89-5422), in which the grain weight was reduced by only 2.6–3.9% following the attack of helminthosporiosis, compared to 27.6–33% losses in susceptible varieties (BL 1135, Sonlika) (Sharma *et al*., 2004). The authors (Sharma *et al.*, 2004a) reported that the resistant varieties SW 89-5422, Yangmai-6, Ning 8201, Chirya 7, Chirya 1, CIGM90.455 were used in the creation of resistant or near-immune lines. However, further field evaluations are needed to confirm the stability of their resistance, as well as the performance of other traits of economic interest (Kumar *et al*., 2019).

Recently, special attention has been paid to research on the genetic control of variability character in plant response to disease. The quantitative nature of resistance to helminthosporiosis is often mentioned in wheat (Bainsla *et al*., 2020; He *et al*., 2020), and communications about the contribution of major genes in governing resistance are also attested. For example, by crossing the resistant genotypes Chirya 3 and MS7 with the common susceptible parent BL 1473 was identified a single dominant gene in the resistant genotypes (Neupane *et al*., 2007). Two-three genes responsible for resistance control have been recorded in some cultivars (Joshi *et al*., 2004). At the same time, in these studies a model of polygenic segregation was found, which indicates that most sources of resistance are governed by multiple genes with minor effects that increase the chances of generating transgressive segregants in the descendant populations (He *et al*., 2020; Singh *et al*., 2018).

The magnitude of heritability (h^2) for resistance to helminthosporiosis varies widely according to different studies – 0.21–0.64 (Sharma *et al*., 2006) and 0.85–0.89 (He *et al*., 2020). However, moderate to high heritability was found in most studies, thus providing a good opportunity to select resistant genotypes in breeding programs.

In connection with the above, the purpose of the research consisted in establishing the influence of *D. sorokiniana* culture filtrates on the variability and the capacity of hereditary transmission of the growth and development characters of common wheat at early stages of development.

MATERIAL AND METHODS

Ten common winter wheat genotypes with high productivity and grain quality indices were involved in the research – the varieties Amor, Centurion, Miranda, Moldova 16 (M16), Moldova 55 (M55), Moldova 66 (M66), Moldova 614 (M614), perspective lines Select/BT-43-42/Select (S/BT/S), Mirgorod/Odeschi27/M79 (M/Od.27/M79), Moldova 79/M/Od.27 (M79/M/Od.27).

The *D. sorokiniana* strains (3) were isolated from the basal part of the wheat stem with signs of root rot on the nutrient medium PDA (Potatoe Dextrose Agar). The pathogen species was identified by macro- and microscopic analysis (Fig. 1) according to the mycological determinant (Barnett, Hunter, 1998).

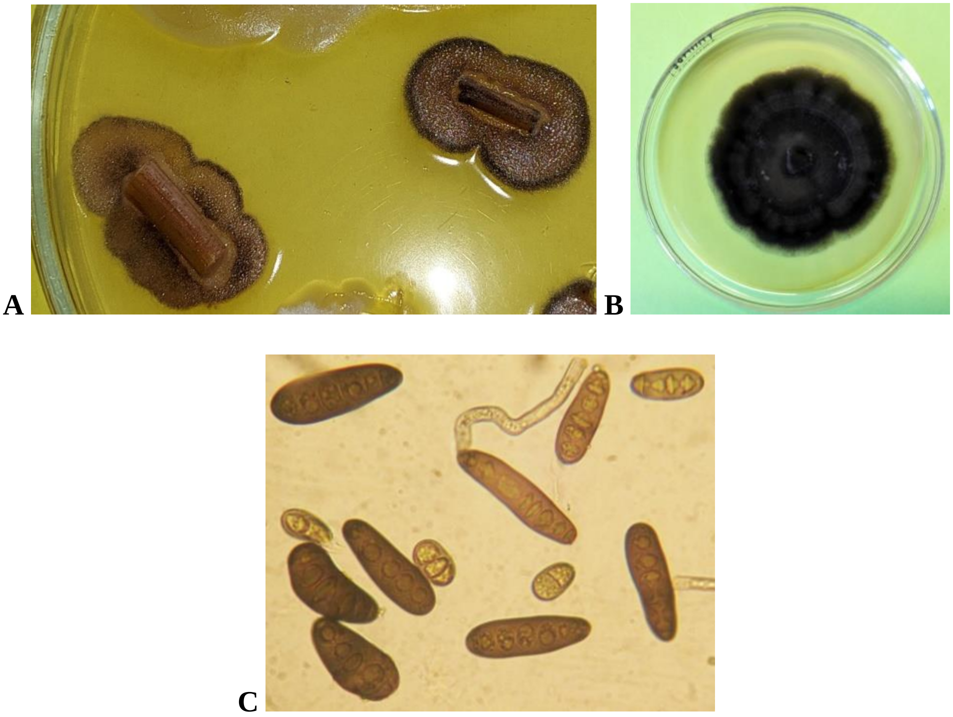


Figure 1. Isolation of the fungus on PDA medium (A), colony (B), conidia of *D. sorokiniana* (C) (300×).

The culture filtrates (CF1, CF2, CF3) of 3 *D. sorokiniana* isolates were prepared by: i) inoculating the mycelium in the Cszapek-Dox liquid nutrient medium containing the following components (g/l distilled water): $NaNO_3$ – 3.0; K_2HPO_4 – 1.0; $MgSO_4 \bullet 7H_2O$ – 0.5; KCl – 0.5; $FeSO_4 \bullet 7H_2O$ – 0.01; sucrose – 30.0 (Methods of experimental mycology, 1982); ii) cultivation for 21 days at a temperature of 23–24°C.

Wheat grains were treated for 18 hours with CF, after which they were rinsed in distilled water, placed in Petri dishes on filter paper moistened with distilled water and cultivated for 6 days at 18–19°C.

Germination (%), embryonic radicle length (mm), stem length (mm), seedling length (cm), vigor index (*germination, % x seedling length, cm*) served as indices of plant reaction to FC treatment.

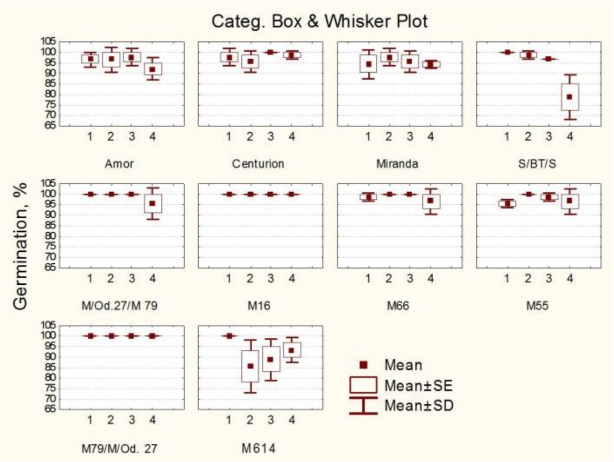
The particularities of the plant – pathogen interaction were established by factor analysis – a multidimensional procedure that highlights the role of independent factors in the variability of the dependent factor (Sharifi *et al*., 2018). The study of heritability, variability and genetic progress is of great importance for elucidating the role of the genotype factor in the formation of the phenotype and establishing the chances of success in using one or another character in the breeding process to create the new variety of plants (Bello *et al*., 2012; Taneva *et al*., 2019). To quantify the variability, the degree of heritability and the genetic advance, the following were calculated: σ^2_g – genetic variance, σ^2_{ph} – phenotypic variance, GCV,% – genotypic coefficients of variation, %, PCV,% – phenotypic coefficients of variation, %, h^2 – heritability in broad sense and GA,% – genetic advance, % according to the authors (Balkan, 2018).

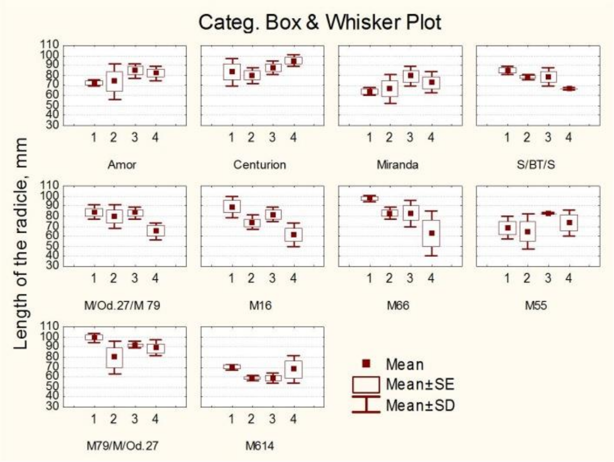
Classification methods that objectively identify the degree of similarity or difference between genotypes have made a considerable contribution to research on plant genetic diversity. For this purpose, two methods of cluster analysis were applied: agglomerative-iterative, with the construction of distribution dendrograms, and centroid by *k*-means method, both of which have been successfully used in genetic and plant breeding research (Ravishanker *et al*., 2013; Koij, Saba, 2015).

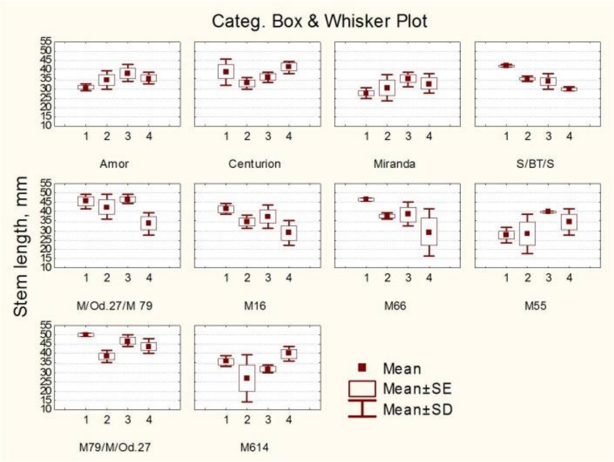
In the *k*-means analysis, 3 clusters were programmed according to the possible values – small, medium, large of the characters under study. The data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSION

The obtained data demonstrated that *D. sorokiniana* culture filtrates did not significantly influence the germination of wheat grains. Only the S/BT/S line showed a deviation of 21.1% compared to the control under the influence of CF3 (Fig. 2).


Categ. Box & Whisker Plot
Germination, %
105 100 95 90 85 80 75 70 65
1 2 3 4
Amor
Centurion
Miranda
S/BT/S
M/Od.27/M 79
M16
M66
M55
M79/M/Od. 27
M614
Mean
Mean±SE
Mean±SD


Categ. Box & Whisker Plot
Length of the radicle, mm
110 100 90 80 70 60 50 40 30
1 2 3 4
Amor
Centurion
Miranda
S/BT/S
M/Od.27/M 79
M16
M66
M55
M79/M/Od.27
M614
Mean
Mean±SE
Mean±SD


Categ. Box & Whisker Plot
Stem length, mm
55 50 45 40 35 30 25 20 15 10
1 2 3 4
Amor
Centurion
Miranda
S/BT/S
M/Od.27/M 79
M16
M66
M55
M79/M/Od.27
M614
Mean
Mean±SE
Mean±SD

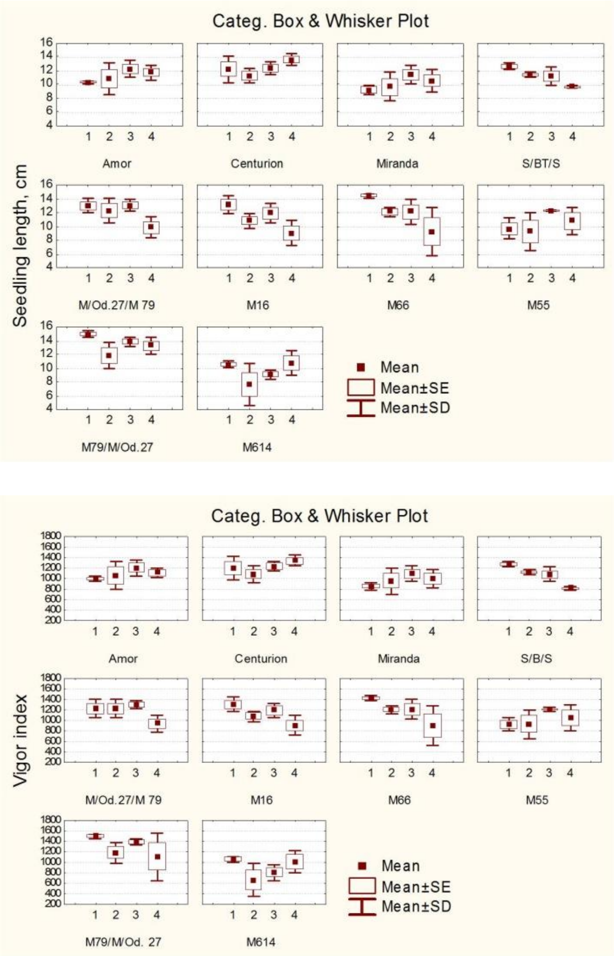


Figure 2. The influence of *D. sorokiniana* culture filtrates on the growth characteristics of common wheat seedlings. *Horizontally*: 1 – Control, 2 – CF1, 3 – CF 2, 4 – CF 3.

In the case of ***embryonic radicle*** growth, both lack of reaction and mild stimulation (Amor, Centurion, Miranda) were found, but also inhibition of varying intensity under the influence of some CFs. For example, on the S/BT/S line under the influence of the 3 CFs, the deviations from the control varied within the limits of -7.7 ... -21.5%. Significant decreases in radicle length were recorded especially when treated with CF3: M/Od.27 (-22.6%), M16 (-30.8%), M66 (-35.4%).

Regarding the ***stem length***, a similar picture to the radicle reaction was observed: lack of reaction/slight stimulation in the first 3 genotypes, inhibition of different intensity in the S/BT/S line: -15.9 ... -29.0%; significant $p<0.05$) under the influence of CF3 in genotypes M/Od.27 (-26.1%), M16 (-30.9%), M66 (-37.4%) ($p<0.05$).

As natural, the reaction of the radicle and stem was reflected on the length of the intact plant, for which similar effects of *D. sorokiniana* CF were recorded.

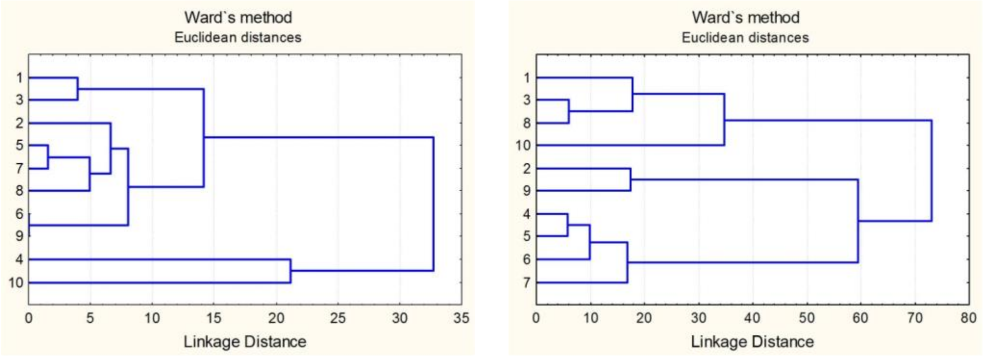
The vigor index demonstrated a relative stability of the first 3 genotypes under the influence of CFs, as in the case of germination and the length of the intact seedling on which it depends. The strongest inhibitory effect was registered in the case of CF3: L S/BT/S (-35.8%), M/Od.27 (-23.1%), M16 (-30.8%), M66 (-36.6%) (Fig. 2).

The classification of wheat genotypes based on the similarity of reaction to the pathogen demonstrated a pronounced specificity for each studied character. For example, genotypes 1, 3, 8 formed a common cluster in the case of radicle length, stem length, vigor index similarities, but separated into different clusters in the case of germination and seedling length. Stable clusters of genotypes for all characters were not found, which denotes the manifestation of a pronounced specific plasticity of growth organs in response to *D. sorokiniana* (Fig. 3).

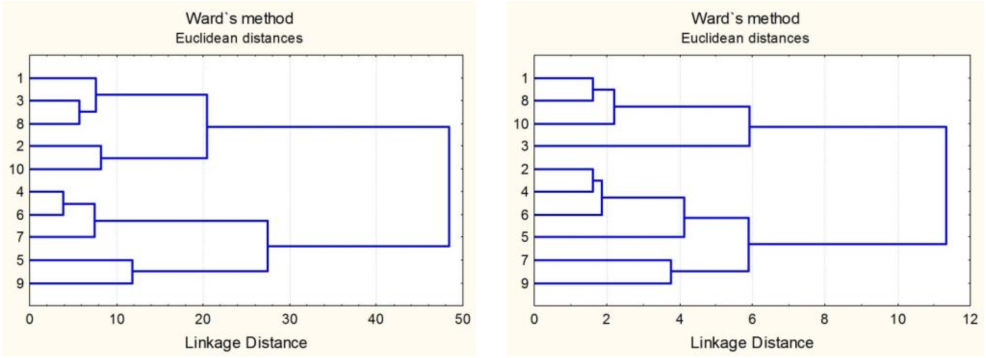
By *k*-means analysis, it was found that only in the case of germination in the control variants and in some variants with CF the ratio of the intercluster variance to the intracluster variant was less than 1 (0.21), which denotes the lack of differentiation of the genotypes into distinct clusters. Thus, we find that CF1 did not show the ability to separate genotypes in the case of seedling length, CF2 – for radicle length, stem length, vigor index, and CF3 – for stem length and seedling length. The phenomenon denotes the lack of a specific interaction of the mentioned CF with certain organs of the wheat genotypes (Table 1).

The classification of genotypes based on the vigor index demonstrated that the best differentiation ability in clusters was exhibited by CF1 and CF2. The mean of the vigor index for cluster 1 decreased significantly compared to the control in variants CF1, CF2 and especially under the influence of CF3 (Fig. 4). Cluster 1 consisted of 6 genotypes – Amor, L S/BT/S, L M/Od.27, M16, M66, M79, cluster 2 – Miranda, M 55, M 614, cluster 3 – Amor, Centurion. Of interest are the Amor and Centurion genotypes from cluster 3, whose average was practically at the level of the control in the CF1 variant (-2.7%), but increased by 10.7% and 11.7% compared to the control in the CF2 and CF3 action.

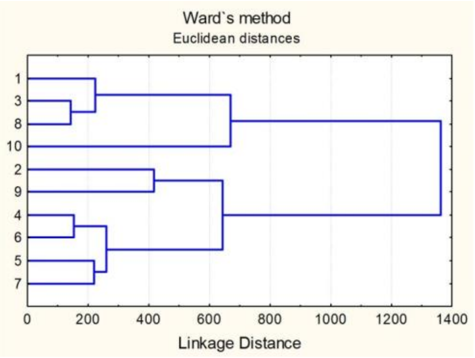
The analysis of the weight of phytopathosystem components in the reaction of plant growth characters to the pathogen demonstrated that in most cases the role of the plant genotype was decisive. Thus, for germination, radicle length, stem length, plant length, the weight of the genotype in the source of variation was 38.2; 44.2; 45.0; 42.0%, respectively. The weight of the isolate factor was also recorded with a not too big difference: germination – 34.3%, radicle length – 37.2%, stem length – 34.1%, plant length – 38.8%. In the case of the vigor index, a higher influence of the isolate was found – 42.6%, with the genotype factor accounting for 37.5%. The role of *genotype x isolated* interactions is not negligible, the weight of which varied depending on the character within the limits of 12.3–19.3% ($p<0.5$) (Table 2).



Germination *Length of the radicle*



Length of the stem *Length of the seedling*



Vigor index

Figure 3. Dendrograms of the wheat genotypes distribution based on the reaction to *D. sorokiniana* culture filtrates. *Vertically:* 1 – Amor, 2 – Centurion, 3 – Miranda, 4 – L S/BT/S, 5 – L M/Od.27/M79, 6 – M16, 7 – M66, 8 – M55, 9 – M79/M/Od.27, 10 – M614.

Table 1

Analysis of intra- and intercluster variance in the reaction of wheat genotypes to *D. sorokiniana* culture filtrates

Character	**Variant**	**Intercluster variance (1)**		**Intracluster variance (2)**	**df**	**F**	**p**	**Ratio 1/2**
Germination	Control	6,806	2	32,519	7	0,83	0,51	0,21
	CF1	156,305	2	22,839	7	23,95	0,00	6,84
	CF2	92,670	2	17,999	7	18,02	0,00	5,15
	CF3	287,027	2	53,269	7	18,86	0,00	5,39
The length of the radicle	Control	1050,542	2	278,628	7	13,20	0,00	3,77
	CF1	414,764	2	167,458	7	8,67	0,01	2,48
	CF2	228,854	2	441,288	7	1,82	0,23	0,52
	CF3	1001,784	2	139,500	7	25,13	0,00	7,18
The length of the stem	Control	515,326	2	59,055	7	30,54	0,00	8,73
	CF1	133,134	2	79,847	7	5,84	0,03	1,67
	CF2	64,686	2	156,355	7	1,45	0,30	0,41
	CF3	87,583	2	165,593	7	1,85	0,23	0,53
The length of the seedling	Control	29,441	2	5,702	7	18,07	0,00	5,16
	CF1	10,647	2	14,232	7	2,62	0,14	0,75
	CF2	10,807	2	8,509	7	4,45	0,06	1,27
	CF3	4,038	2	11,640	7	1,21	0,35	0,35
Vigor Index	Control	314872,3	2	89205,5	7	12,35	0,01	3,53
	CF1	190748,8	2	66394,5	7	10,06	0,01	2,87
	CF2	76267,5	2	138044,4	7	1,93	0,22	0,55
	CF3	124118,4	2	74540,8	7	5,83	0,03	1,67

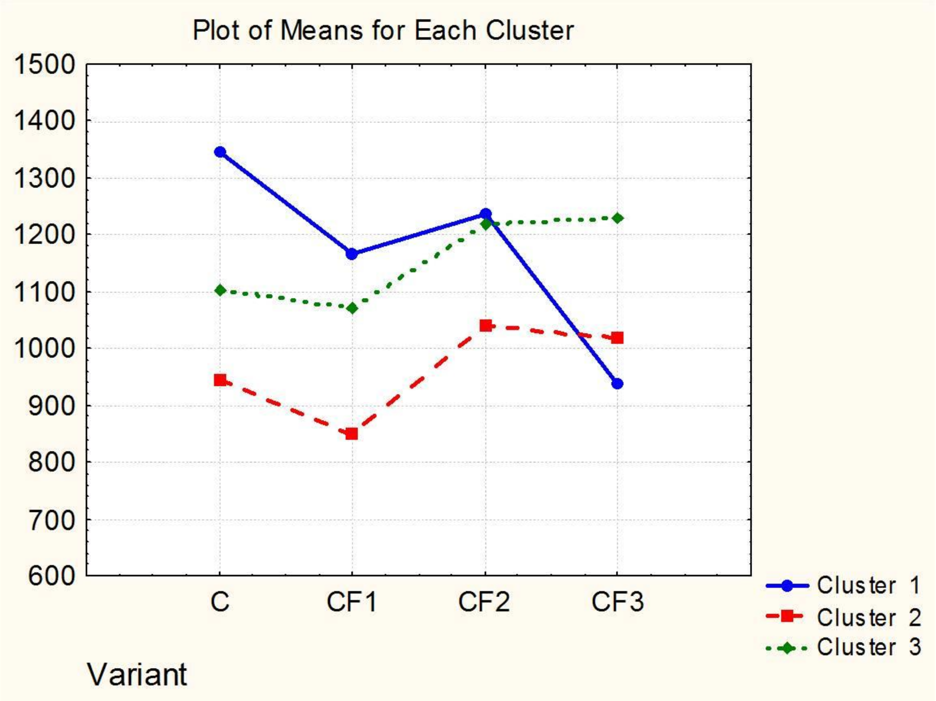


Figure 4. Classification of wheat genotypes based on the vigor index, when interacting with *D. sorokiniana* culture filtrates. *Vertically, on the left* – the vigor index.

The genotypic (σ^2_g) and phenotypic (σ^2_{ph}) variances recorded a differentiated magnitude of the researched characters: 4.48 ... 47643 and 6.76 ... 79645.67, respectively, the highest values being reported for the vigor index, and the smallest – for the length of the seedling. It should be noted that although a different level of these parameters was registered for the characters under study, the ratio σ^2_g/σ^2_{ph} was similar in all cases, which determined the obtaining of values within rather narrow limits for the heritability coefficient in a broad sense (h_2): 0.55–0.69.

Calculation of GCV (%) demonstrated the existence of low level genetic control for germination (4.98%), medium – radicle length, seedling length, vigor index (18.0–19.7%), and moderately high – length stem (21.3%). It should be noted that the genetic advantage (GAM, %) recorded low values (6.6%) for germination, but high values for the other characters (23.3–28.7%) (Table 3).

Table 2

Contribution of genotype and *D. sorokinina* isolate factors to the growth capacity of common winter wheat

Source of variation	**Freedom degree**	**Mean sum of squares**	**F**	**p**	**Share in the source of variation, %**
Germination					
Wheat genotype	9	89*	4.63	0.000	38.20
Fungus isolate	3	80*	4.14	0.009	34.34
Genotype x isolate	27	45*	2.31	0.002	19.31
Random effects	80	19			8.16
The length of the radicle					
Wheat genotype	9	678.6*	6.946	0.000	44.19
Fungus isolate	3	571.0*	5.844	0.001	37.18
Genotype x isolate	27	188.4*	1.929	0.013	12.27
Random effects	80	97.7			6.36
The length of the stem					
Wheat genotype	9	210.2*	7.760	0.000	45.04
Fungus isolate	3	159.2*	5.878	0.001	34.11
Genotype x isolate	27	70.2*	2.592	0.000	15.04
Random effects	80	27.1			5.81
The length of the seedling					
Wheat genotype	9	15.71*	6.888	0.000	42.04
Fungus isolate	3	14.49*	6.356	0.001	38.77
Genotype x isolate	27	4.89*	2.145	0.005	13.09
Random effects	80	2.28			6.10
Vigor Index					
Wheat genotype	9	174933	5.466	0.000	37.48
Fungus isolate	3	198781	6.212	0.001	42.59
Genotype x isolate	27	61044	1.908	0.014	13.08
Random effects	80	32002			6.86

*- $p<0.05$.

Table 3

Heritability and variability of growth and development characters
of common winter wheat in interaction with *D. sorokiniana*

Parameter	**Germination**	**The length of the radicle**	**The length of the stem**	**The length of the seedling**	**Vigor Index**
σ^2_G	23,33	193,63	61,03	4,48	47643
σ^2_{Ph}	42,33	291,33	88,13	6,76	79645,67
h^2	0,55	0,665	0,693	0,66	0,60
GCV, %	4,98	17,95	21,34	18,58	19,74
PCV, %	6,70	22,01	25,64	22,83	25,52
PCV – GCV, %	1,72	4,06	4,30	4,25	5,78
GAM, %	6,60	23,32	28,66	24,48	25,88

Thus, the study of the growth characteristics of common wheat seedlings in interaction with isolates of the fungus *D. sorokiniana* and the application of multidimensional statistical procedures allowed the elucidation of the role of genotypic and biotic factors in the variability and heritability of the character of resistance to the pathogen.

CONCLUSIONS

The treatment of the grains of 10 common winter wheat genotypes with culture filtrates of *3 D. sorokiniana* isolates demonstrated the manifestation of specific plant reactions (lack of reaction, inhibition, stimulation) depending on the genotype, growth organ, fungal isolate.

Cluster analysis (dendrograms, *k*-means methods) based on the reaction to 3 *D. sorokiniana* isolates highlighted different classifications of wheat genotypes according to the organ tested, which indicates their phenotypic plasticity. Two genotypes were identified – Amor and Centurion with capacity for positive interaction with *D. sorokiniana* isolates, which led to the significant increase (10.7–11.7%) of an integral index of growth and development – the vigor index.

The factorial analysis demonstrated that in the reaction of common wheat to *D. sorokiniana* isolates, the weight of the genotype factor was 38.2; 44.2: 45; 42%, and isolated – 34.3%, 37.2%, 34.1%, 38.8%, respectively, of germination, radicle length, stem length, seedling length. In the case of the vigor index, a higher influence of the isolate was recorded – 42.6%, with the genotype factor accounting for 37.5%. The contribution of *genotype x isolate* interactions varied depending on the character within the limits of 12.3–19.3% ($p<0.5$).

The pronounced association of the heritability coefficient (h^2) and the genetic advantage with fairly high values for the radicle length, the stem length, the plant length, the vigor index reveal: i) the suitability of the characters as selection factors; ii) the additive control of resistance, which offers chances of success in creating resistant genotypes to *D. sorokiniana*.

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