CAUSATIVE AGENTS OF ROOT ROT IN WINTER WHEAT

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Abstract

Knowledge of the composition of fungal species that cause root rot in common wheat is of great importance for the development of correct protection measures and serves as methodological support in pathogen resistance screening systems. In our research, 14 fungal species involved in the development of the disease in 2024 under the conditions of the Republic of Moldova were recorded. An increase in the frequency of the fungi *Fusarium culmorum* (24%) and *F. equiseti* (31.8%) was reported. Cluster analysis demonstrated the important role of the variety in the frequency of fungal spread. It was found that one of the most widespread causative agents of root rot – *Fusarium oxysporum* can cause both inhibition and stimulation of plant growth, as well as a change in the spectrum of phenotypic classes in segregating F_2 populations. The orientation of the crossing of the parents when creating F_1 wheat hybrids significantly influences the reaction of plants to the pathogen, but also their variability in terms of growth and development traits.

Keywords: wheat, fungi, root rot, variability.

In the context of increasingly alarming climate change over the last two decades, issues related to crop plant resistance to pathogens are becoming more relevant. Sudden fluctuations in temperature and humidity cause serious physiological disturbances in plants, making them increasingly susceptible to various fungal diseases.

Although common wheat (*Triticum aestivum* L.) is adapted to various growing conditions, it is vulnerable to soil-borne pathogens that cause various diseases, one of the most widespread being root rot – a major problem worldwide. The disease manifests itself through rotting of the root system in the soil, the base of the stem, wilting of plants, weight loss of plants and grains. Along with this, many pathogens are active producers of mycotoxins, thus contributing to the pollution of grains and increasing their toxicity. All these manifestations lead to plant weakness and crop losses (Kiecana I. *et al*, 2016). Root rot is a unique disease, as it is caused by different causative agents. Pathogens are extremely labile to climate changes – temperature, drought, humidity and soil acidity, but also to changes in agrotechnical conditions and the genotypic structure of the crops.

Recently, some authors have drawn attention to the important role of increasing the concentration of CO_2 in the atmosphere on the increase in the pathogenicity of some fungi involved in the development of the disease, such as *Fusarium equiseti* (Gilardi G. *et al*, 2018), which is usually considered a species with weak virulence.

Establishing of the composition of fungal species that cause one or another disease in crop plants is of great importance for the development of correct protection measures and serves as methodological support in pathogen resistance screening systems.

The purpose of the research was to establish the composition of fungal species that cause root rot in common wheat under the conditions of the Republic of Moldova and the influence of the most widespread ones on plant variability in segregating populations.

MATERIAL AND METHOD

The study material was winter wheat plants grown on the experimental sector of the Institute of Genetics, Physiology and Plant Protection, Moldova State University.

The samples presented individual selections from common wheat varieties approved in the Republic of Moldova and Romania. The research was carried out in the conditions of 2024.

The assessment of the degree of root rot attack was carried out at the technical ripening stage of the berries by examining the affected surface at the base of the stem on a 5-step scale: 0 – healthy plant, 0.1 – punctate necrosis, 1 – 5-10%, 2 – 20-50%, 3 – 60-100% of the surface.

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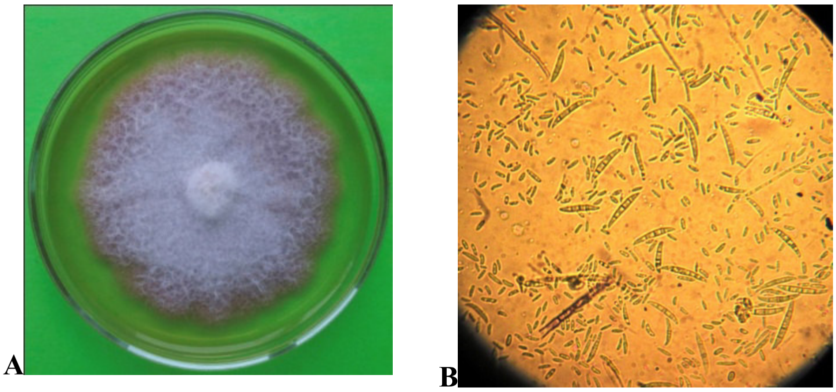
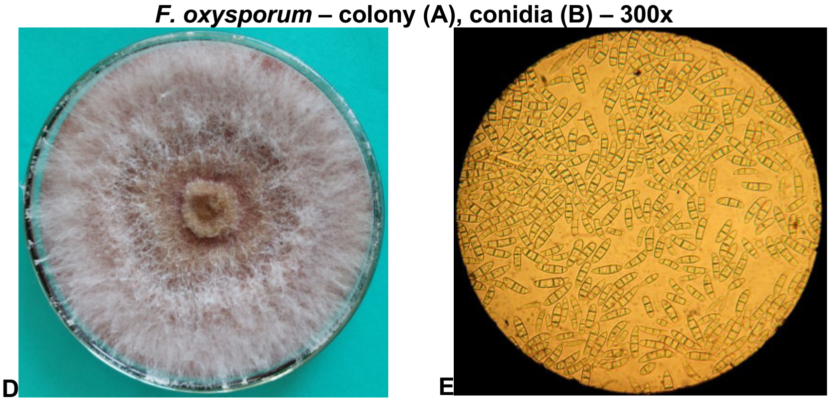
Figure 1 **Appearance of isolated fungi from the base of the wheat stem on PDA medium**



***F. oxysporum* – colony (A), conidia (B) – 300x**



***F. culmorum* – colony (A), conidia (B) – 300x**

Figure 2 **Macro- and microscopic aspects of some fungi isolated from the base of the wheat stalk**

Isolation of pathogens from plants with disease symptoms was performed on the solid nutrient medium PDA (Potatoe Dextrose Agar) (figure 1), and determination of the fungal species – based on macro- and microscopic analyses (Barnet H.L., Hunter B.B., 1998) (figure 2).

The Margalef diversity index of fungal species was calculated according to the formula (S-1/lnN), and the Berger-Parker dominance index: N max/N total (Margalef R., 1968; Caruso T. et al, 2007; Kitikidou, K. et al, 2024).

The F. oxysporum culture filtrate was prepared on the basis of Cszapek liquid nutrient medium (Methods of experimental mycology, 1982).

The obtained data were statistically processed in the STATISTICA 7 software package through variance analysis, cluster analysis (dendrograms, k-means), and plant distribution histograms.

RESULTS AND DISCUSSIONS

It was found that the degree of root rot attack on the 5 wheat genotypes under study was 1.07 ... 1.41, so the attack was of medium level (*table 1*). The frequency of isolated fungal species varied within wide limits: *F. oxysporum* (6.2%), *F. oxysporum* var. *orthoceras* (6.7%), *F. solani* (1,7%), *F. moniliforme* var. *lactis* (0.6%), *F. culmorum* (24%), *F. equiseti* (31.8%), *F. acuminatum* (5%), *F. graminearum* (0.6%), *Drechslera sorokiniana* (9.5%), *D. avenae* (4.5%), *D. biseptata* (1.1%), *Rhizoctonia solani* (1.1%), *Sclerotium rolfsii* (5%), *Mycelia sterilia* (2.2%).

F. oxysporum/F. oxysporum var. *orthoceras* were recorded in all samples. It is worth noting that *F. oxysporum* is one of the most widespread and virulent fungi that causes root rot in cereal crops, including wheat (Özer G. *et al*, 2023). In the Republic of Moldova, *Fusarium oxysporum* var. *orthoceras* are the more widespread variety (Lupaşcu G. *et al,* 2008; Lupaşcu G., 2020). Lately, in the conditions of the Republic of Moldova, there has been a decrease in the incidence of this fungus as a result of ecological imbalances that favor the development and spread of others.

Of interest is the rather high frequency (24%) of the fungus *F. culmorum* (*table 2*), which in previous years was not isolated at all from wheat plants with signs of root rot or was recorded in very limited numbers. The increase in the incidence of this fungus is probably due to the frequent association of dry periods with low nighttime temperatures in the conditions of the Republic of Moldova. According to some data, *F. culmorum* is dominant in colder and semi-arid areas, such as North Dakota (USA) (Shrestha S. et al, 2021), northern, central and western Europe (Wagacha J.M., Muthomi J.W., 2007).

Table 1

Composition of fungal species – causal agents of root rot in wheat

Genotype	Root rot, degree		Fungus species	Isolates, number	Frequency, %
	$x±m_x$	σ			
Moldova 16	1,07±0,12	0,67	*F. oxysporum* var. *orthoceras*	4	11,4
			F. solani	1	2,9
			F. culmorum	20	**57,1**
			F. equiseti	1	2,9
			F. acuminatum	3	8,6
			Drechslera avenae	3	8,6
			D.biseptata	1	2,9
			Rhizoctonia solani	2	5,7
			Total:	***35***	***100***
Amor	1,23±0,17	0,91	*F. oxysporum* var. *orthoceras*	2	7,1
			F. solani	1	3,6
			F. culmorum	12	**42,9**
			F. graminearum	1	3,6
			F. equiseti	7	**25,0**
			Sclerotium rolfsii	1	3,6
			Mycelia sterilia	4	14,3
			Total:	***28***	***100***
Miranda	1,33±0,12	0,68	*F. oxysporum* var. *orthoceras*	1	3,1
			F. moniliforme var. *lactis*	1	3,1
			F. equiseti	23	**71,9**
			F. acuminatum	5	**15,6**
			Sclerotium rolfsii	2	6,3
			Total:	***32***	***100***
Moldova 66	1,41±0,12	0,66	*F. oxysporum*	11	**21,6**
			F. culmorum	10	**19,6**
			F. equiseti	13	**25,5**
			F. acuminatum	1	2,0
			D. sorokiniana	10	**19,6**
			D. avenae	5	9,8
			D.biseptata	1	2,0
			Total:	***51***	***100***
Centurion	1,12±0,14	0,80	*F. oxysporum* var. *orthoceras*	5	15,2
			F. solani	1	3
			F. culmorum	1	3
			D. sorokiniana	7	21,2
			F. equiseti	13	**39,4**
			Sclerotium rolfsii	6	18,2
			Total:	***33***	
			TOTAL for 5 genotypes:	***179***	

Table 2

Frequency of fungal species – causal agents of root rot in wheat

Nr.	Species	Isolates, number	Frequency, %
1	*Fusarium oxysporum*	11	6,15
2	*F. oxysporum* var. *orthoceras*	12	6,70

3	*F. solani*	3	1,68
4	*F. moniliforme* var. *lactis*	1	0,56
5	*F. culmorum*	**43**	**24,02**
6	*F. equiseti*	**57**	**31,84**
7	*F. acuminatum*	9	5,03
8	*F. graminearum*	1	0,56
9	*Drechslera sorokiniana*	17	9,50
10	*D. avenae*	8	4,47
11	*D. biseptata*	2	1,12
12	*Rhizoctonia solani*	2	1,12
13	*Sclerotium rolfsii*	9	5,03
14	*Mycelia sterilia*	4	2,24
	Total:	***179***	***100***

It was found that the Margalef diversity index was 2.51, and the Berger-Parker dominance index: 0.32 for the species *F. equiseti*.

The dendrogram of the distribution of fungal species based on their frequency in the 5 wheat varieties demonstrates the existence of similarities but also differences, which led to the formation of clusters, different in size according to the number of members (species) and level of aggregation / similarity (*figure 3*).

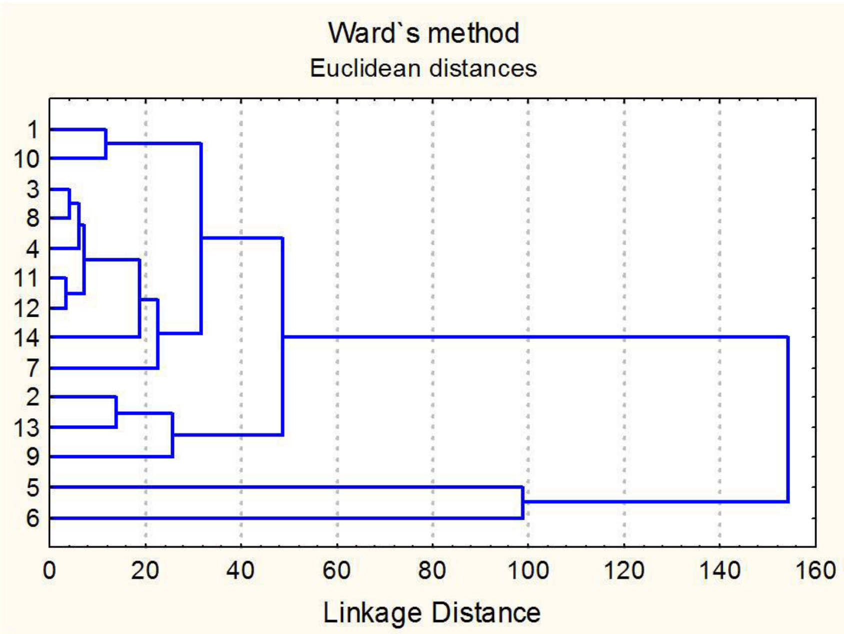


Figure 3 Dendrogram of fungal species distribution based on frequency similarity in wheat plants

Vertically: 1 – *Fusarium oxysporum,* 2 – *F. oxysporum* var. *orthoceras,* 3 – *F. solani,* 4 – *F. moniliforme* var. *lactis,* 5 – *F. culmorum* , 6 – *F. equiseti,* 7 – *F. acuminatum,* 8 – *F. graminearum,* 9 – *Drechslera sorokiniana,* 10 – *D. avenae,*11 – *D. biseptata,*12 – *Rhizoctonia solani,* 13 – *Sclerotium rolfsii,*14 – *Mycelia sterilia*

The calculation of inter- and intracluster distances by the centroid clustering method (k-means) demonstrated that, with the exception of the Moldova 66 variety, the distance between clusters was much greater than within it, which denotes the successful separation/classification of fungal species according to frequency. The ratio of intercluster to intracluster distance demonstrated that the highest capacity for differentiating fungi based on frequency was recorded by Moldova 16 and Miranda (*table 3*).

Table 3

Analysis of variance

Genotip	Between SS (1)	df	Within SS (2)	df	F	Signif. p	Variance ratio 1/2
Moldova 16	2821.64	2	135.57	11	114.47	0.00	20.8
Amor	1836.10	2	207.38	11	48.70	0.00	8.9
Miranda	4551.01	2	240.58	11	104.04	0.00	19.0
Moldova 66	570.78	2	702.68	11	4.7	0.04	0.81
Centurion	1123.55	2	744.24	11	8.0	0.01	1.5

From the first genotype were extracted the most isolates *F. culmorum* (57.1%), and from the 2nd – the most isolates *F. equiseti* (71.9%). Therefore, it could be assumed that wheat varieties exhibit some specificity of susceptibility to root rot causative agents.

Recently, there has been a growing interest in plant-fungal interactions that can have strong effects on plant density, both through direct actions on plant performance/vigor and indirectly – on fungal/plant competition, but also through selective promotions of some species. Most of the evidence demonstrating certain links between plant abundance derives from direct fungal effects on initial growth, but with little evidence linking fungal effects on plant-plant interactions in intact communities (Bennett J., Cahill J.F., 2016).

Our research on the influence of treating wheat grains with CF *F. oxysporum* for 18 hours demonstrated that in the segregating F_2 Moldova 11 x Centurion population, the pathogen produced inhibition of embryonic radicle growth by 17.3% (*figure 4*).

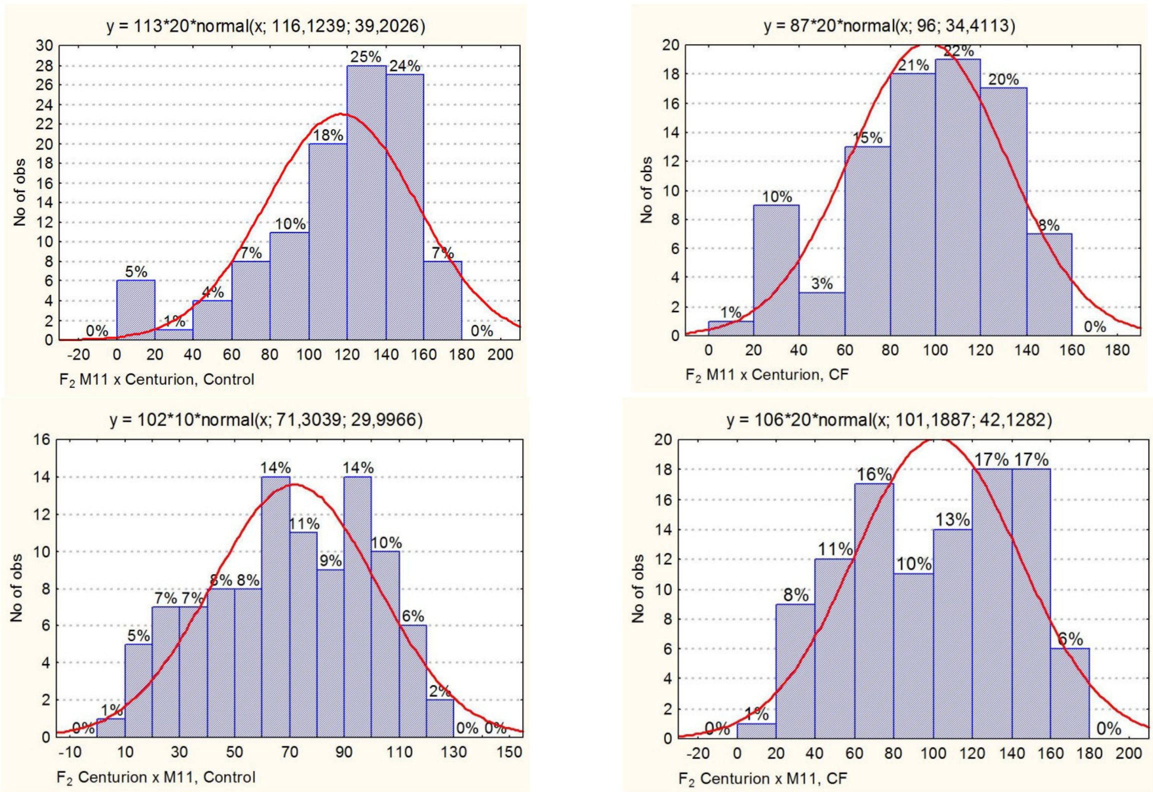


Figure 4 Influence of the *F. oxysporum* culture filtrate on the distribution of wheat plants into phenotypic classes based on embryonic root length

At the same time, changes in the spectrum of phenotypic classes were observed under the influence of the pathogen. Thus, in the CF variant, the "160-180 mm" class, recorded in the control variant, was not found.

It is worth noting that in the F_2 population Centurion x Moldova 11, unlike the previous population, CF stimulated the growth of the embryonic radicle, the length of which was 41.9% greater than in the control variant. At the same time, under the influence of CF in the spectrum of phenotypic classes, 2 new classes appeared: "140-160 mm" and "160-180 mm", which together constituted 23% of the general population.

The obtained data demonstrate that the phenomenon of *plant – F. oxysporum* interaction strongly influences the effect of the pathogen on plant growth, but also on their variability. At the same time, the direction of crossing parental forms to obtain F_1 hybrids significantly influences the reaction of wheat plants in segregating populations, which is of great importance in the development of strategies for carrying out breeding programs.

CONCLUSIONS

1. Macro- and microscopic research of 179 fungal isolates extracted from common winter wheat plants with signs of rot at the base of the stem established the presence of 14 species/variations – *Fusarium oxysporum* (6.2%), *F. oxysporum* var. *orthoceras* (6.7%), *F. solani* (1,7%), *F. moniliforme* var. *lactis* (0.6%), *F.*

culmorum (24%), *F. equiseti* (31.8%), *F. acuminatum* (5%), *F. graminearum* (0.6%), *Drechslera sorokiniana* (9.5%), *D. avenae* (4.5%), *D. biseptata* (1.1%), *Rhizoctonia solani* (1.1%), *Sclerotium rolfsii* (5%), *Mycelia sterilia* (2.2%).

2. Through cluster analysis methods (dendrogram, *k*-means) it was found that the wheat variety factor is of great importance in the classification of fungal species based on colonization frequency, which signifies some specificity of sensitivity of genotypes for the causal agents of root rot.

3. It was found that the treatment of wheat grains with *F. oxysporum* culture filtrate influences the growth of the embryonic radicle by inhibition or stimulation and the spectrum of phenotypic classes in the F_2 segregating populations. These phenomena are determined by the direction of crossing when obtaining F_1 hybrids, which should be taken into account when carrying out breeding programs for root rot resistance.

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REFERENCES

Barnet H.L., Hunter B.B., 1998 - *Illustrated Genera of Imperfect Fungi*. 4th Edition, APS Press, 218. http://apspress.edu.com

Bennett J., Cahill J.F., 2016 - *Fungal effects on plant–plant interactions contribute to grassland plant abundances: evidence from the field*. Journal of Ecology, 104:755–764.

Caruso T., Pigino G., Bernini F., Bargagli R., Migliorini M., 2007 - The Berger-Parker index as an effective tool for monitoring the biodiversity of disturbed soils: A case study on Mediterranean oribatid (Acari: Oribatida) assemblages. Biodiversity and Conservation, 16:3277-3285.

Gilardi G., Garibaldi A., Gullino M. L., 2018 - *Emerging pathogens as a consequence of globalization and climate change: leafy vegetables as a case study*. Phytopathologia Mediterranea, 57, 1:146−152

Kiecana I., Cegiełko M., Rachoń L., Pastucha A., Wit M., Pojmaj M., 2016 - *The occurrence of fungi on roots and stem bases of Triticum aestivum ssp. spelta L. Thell. grown under two levels of chemical protection and harmfulness of Fusarium graminearum Schwabe to seedlings of selected genotypes.* Acta Agrobot., 69(3):1657.

Kitikidou, K., Milios, E., Stampoulidis, A., Pipinis, E., Radoglou, K., 2024 - *Using Biodiversity Indices Effectively: Considerations for Forest Management. Ecologies*, *5*(1):42-51.

Lupașcu G., 2020 - Putregaiul de rădăcină la grâul comun de toamnă. Chisinău: Print-Caro, 120 p.

Lupaşcu G., Saşco E., Gavzer S., 2008 - *Componenţa speciilor de fungi care produc boli la Triticum aestivum L.* Buletinul A.Ş.M. Seria Ştiinţele vieţii, 2 (305):66-73

Margalef R., 1968 - Perspectives in Ecological Theory. University of Chicago Press, Chicago, IL, p.111.

Methods of experimental mycology, 1982, Kiev: Naukova dumka, 550 c. (Ru).

Özer G., Erper İ., Yıldız Ş., Bozoğlu T., Zholdoshbekova S., Alkan M., Tekin F., Uulu T.E., İmren M., Dababat A., Derviş S., 2023 - *Fungal Pathogens Associated with Crown and Root Rot in Wheat-Growing Areas of Northern Kyrgyzstan*. J. Fungi (Basel). 9(1), 124.

Shrestha, S., Poudel, R. S., Zhong, S., 2021 - *Identification of Fungal Species Associated with Crown and Root Rots of Wheat and Evaluation of Plant Reactions to the Pathogens in North Dakota.* Plant Dis., 105(11):3564-3572.

Wagacha, J.M., Muthomi, J.W., 2007 - *Fusarium culmorum: Infection Process, Mechanisms of Mycotoxin Production and Their Role in Pathogenesis in Wheat*. Crop Protection, 26:877-885