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MANIFESTATION OF RESISTANCE TO SOME PATHOGENIC FUNGI AND PRODUCTIVITY CHARACTERISTICS IN TOMATOES

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Abstract

As a result of the analysis of the reaction of tomato genotypes to the culture filtrates (CF) of Alternaria alternata, Fusarium oxysporum, F. solani fungi, it was found that in the most cases they had a negative influence on seed germination. An inhibition of less than 15% in all FCs studied was recorded in the Flacăra, Mary Gratefully and L 10 B varieties, thus showing interest in the breeding process as possible donors of complex resistance to the seed germination stage. Root and stem growth in the evaluated varieties and lines was most strongly influenced by F. solani and A. alternata. The lowest sensitivity of root and stem to FC was recorded at L 66. The climatic conditions of the 2019 year have significantly influenced the general harvest of tomatoes. The highest productivity was recorded in the varieties Mary Gratefully (31.5 t/ha), Rome (30.3 t/ha) and L 71 lines (32.2 t/ha), that can be successfully used in the breeding when creating new varieties with high adaptability to the pedoclimatic conditions.

Key words: *Alternaria alternata*, *Fusarium spp., productivity, resistance, tomatoes.*

INTRODUCTION

Among the factors that greatly affect the productivity and quality of tomatoes fruit can be mentioned the low temperature, especially at the early stages of ontogenesis (Foolad and Lin 2000; Bralewski et al., 2004; Mihnea, 2006), burning in summer (Sato et al., 2006; Bita, Gerats, 2013; Mihnea and Lupașcu, 2018), root rot and alternariosis fungal diseases (Lupaşcu, Rotaru and Mihnea, 2009; Mihnea, Lupașcu and Gavzer, 2018).

Under the conditions of the Republic of Moldova, lately, in tomatoes are recorded the fungal pathogens *Fusarium* spp. which cause root rot at different stages of development, weakening and wilting of plants and *Alternaria* spp., which is manifested by brown stains of leaves, shoots, fruit and root rot (Lupaşcu et al., 2008; Lupascu et al., 2015; Mihnea, 2017).

The use of advanced technologies, resistant varieties, chemical treatments are considered as basic factors among the effective measures for the management of diseases in tomatoes. The lifespan of resistant varieties, usually recommended for production, is often limited due to the emergence of different pathogens new races that exceed the genes resistance of the cultivated varieties (Amini and Sidovich, 2010).

Varieties and hybrids that are characterized by high productivity stability under different ecological conditions are of great importance for agriculture. Thus, obtaining stable crops at vegetable plants, including tomatoes, reducing losses due to diseases and environmental unfavorable factors can be achieved by creating resistant varieties, with ecological stability and high plasticity (Mihnea, Lupașcu and Gavzer, 2018).

Creating resistant tomato varieties is one of the most effective strategies for controlling fungal diseases (Zhang et al., 2003; Çalış and Topkaya, 2011; Mihnea, Lupascu and Grigorcea, 2017).
The tomatoes genofond of the Institute of Genetics, Physiology and Plant Protection, currently has over 900 samples and consists of samples of local origin, collected from amateur growers; varieties of IGFP involved in tomato selection; varieties purchased from retail sales, which belonged to different seed companies abroad. Currently, the genofond is completed with samples created in the institute, through scientific cooperation and material exchange between scientific institutions and gene banks abroad.
The assessment of plant genetic resources is based on their differentiation according to the most important characteristics, highlighting the forms that have the capacity of hereditary
The aim of our research was to identify the level of resistance of some varieties and tomato lines to the fungal pathogens *Fusarium* spp., *A. alternata* in laboratory conditions and genotypes with high productivity under field conditions.

MATERIAL AND RESEARCH METHODS

As a research material, 6 varieties and 4 tomato lines were served. The lines and varieties Kristina, Florina, Măriuca, Darsirius, are of romanian origin from the Vegetable Research and Development Station Buzau. The experiments were carried out in laboratory and field conditions, on the experimental field of the Institute of Genetics, Physiology and Plant Protection, Republic of Moldova. Resistance to fungal diseases was carried out under laboratory conditions.
The culture filtrates (CF) of the fungi *Fusarium oxysporum, F. solani* and *Alternaria alternata* (isolated from diseased tomato plants) were used, prepared by inoculation of the mycelium in Czapek-Dox liquid medium and further cultivated at 22-24°C for a time for 21 days.
The tomato seeds were treated with CF of the fungi for 18 hours. The seeds kept in distilled water served as a control. Seedlings were grown in Petri dishes on filter paper moistened with distilled water at 22-24ºC for 6 days. As a test index of the plant reaction, important growth and development characteristics of tomatoes were used at the early stage of ontogenesis – germination, root length and stem length.
Cluster analyzes by constructing dendrograms (agglomerative-iterational algorithm, the Ward method), and the *k*-media method were performed (Savary, S. et al., 2010). Within the k-means method, 3 clusters were programmed according to the possible values of the characters: small, medium and high.
Tomatoes were grown by seedling culture in three repetitions according to the standard method (Erşova, 1978). Planting in the field took place in the third decade of May. The data obtained were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

The varieties and lines in the collection that showed a complex of useful characters in 2018 were tested under laboratory conditions to determine the reaction to the culture filtrates of pathogens *F. oxysporum, F. solani* and *A. alternata* based on seeds germination, embryo root and stem length. There was a strong inhibition of seeds germination, growth and development of the embryonic root and stem. It is worth mentioning that the reaction of the plants depended on the genotype, the analyzed character and the fungal species.
As shown from the data presented, the influence of CF on seeds germination in tomato genotypes included in the study was different (Figure 1).
For example, *F. oxysporum* CF inhibited seeds germination by -1.6% ... -30.3%, and *F. solani* by -5.8% ... -46.4%. Under the influence of *A. alternata* CF inhibition was -10.0% ... -44.9%. Significant inhibition was recorded in Florina variety (-30.3%) and lines L 66 (-19.4%), L 11 (-16.4%) under the influence of *F. oxysporum* CF.

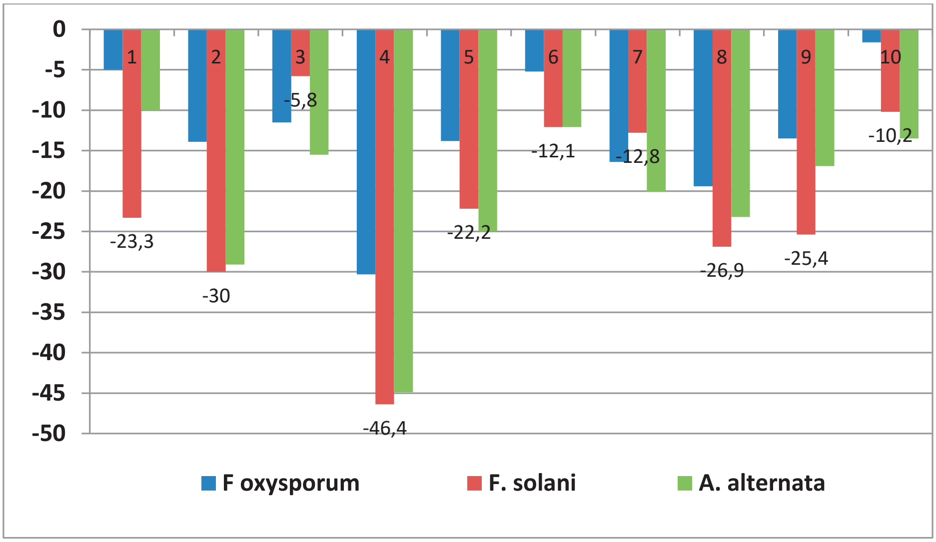

Figure 1. The influence of culture filtrates on seeds germination (%)
of perspective lines and tomato varieties:

1. Roma; 2. Pontina; 3. Flacara; 4. Florina; 5. Mariuca; 6. L 10 B; 7. L 11; 8. L 66; 9. L 71; 10. Mary Gratefully

In the variant with *F. solani* CF strong character inhibition was observed in Florina, Pontina, Roma varieties, L 66, L 71 - 46.4, 30.0, 23.3, 26.9, 25.4%, and insignificant inhibition in Flacara and Mary Gratefully - 5.8 and 10.2%, respectively. Under the influence of *A. alternata* CF significant inhibition was found in the varieties Florina (44.9%), Pontina (29.1%), Măriuca (25.0%) and L 66 lines (23.2%), L 11 (20.1%). An inhibition of less than 15% in all evaluated CFs was recorded in the Flacara, Mary Gratefully and L 10B varieties, thus showing interest in breeding programs as possible sources of resistance to these fungal pathogens.

It was found that in the case of the root, the genotypes showed quite high sensitivity to CF (Figure 2). Thus, the above-mentioned culture filtrates inhibited root growth within the limits of 35.0 … 82.0%. The evaluated genotypes were most strongly influenced by *F. solani* and *A. alternata*, the average values relative to the control varying within the limits -46.9 ... -80.3% and -47.1 ... -82.0%, respectively. There were strong repressions at L 71, Florina, L 66, Flacara variant with *F. oxysporum* CF. In 8 of the 30 cases there was an inhibition of the growth of the embryonic root within the limits 35.0 ... 47.9%. Therefore, no genotypes with low sensitivity were attested, but the lowest sensitivity to the CF studied was recorded at L 66.

In the case of stem length, a wider amplitude of variability was identified in response to the CF fungus (Figure 3). Stem inhibition in relation to the control ranged within the limits -48.3 … -71.1% in *F. oxysporum*, -55.0.2… -84.7% - *F. solani*, -61.2… -85,7% - *A. alternata*. As in the case of the root, the genotypes were most strongly influenced by *F. solani* and *A. alternata*. For example, in the variant with *F. solani* CF, inhibition of more than 60.0% was observed in all genotypes, except for the L 66 line, where the growth of the strain was suppressed by 55.0%.

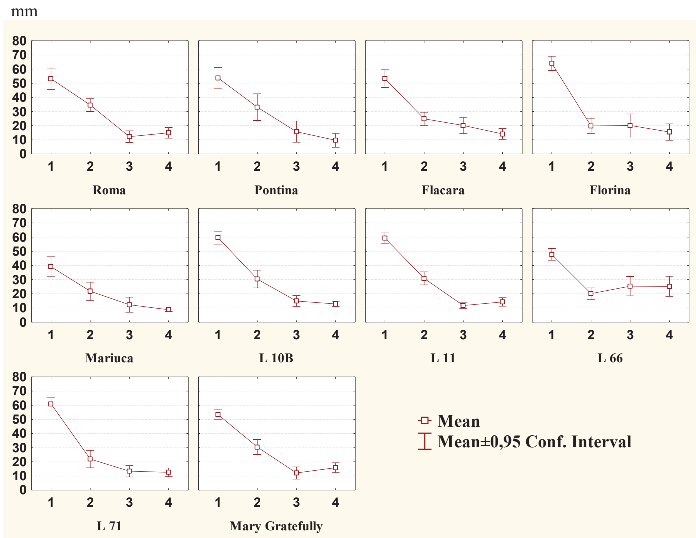

Figure 2. Influence of *F. oxysporum, F. solani, A. alternata* culture filtrates on root growth in tomato seedlings
Vertical: the root length
Horizontal: H_2O (control); 2. *F. oxysporum*; 3. *F. solani*; 4. *A. alternata*

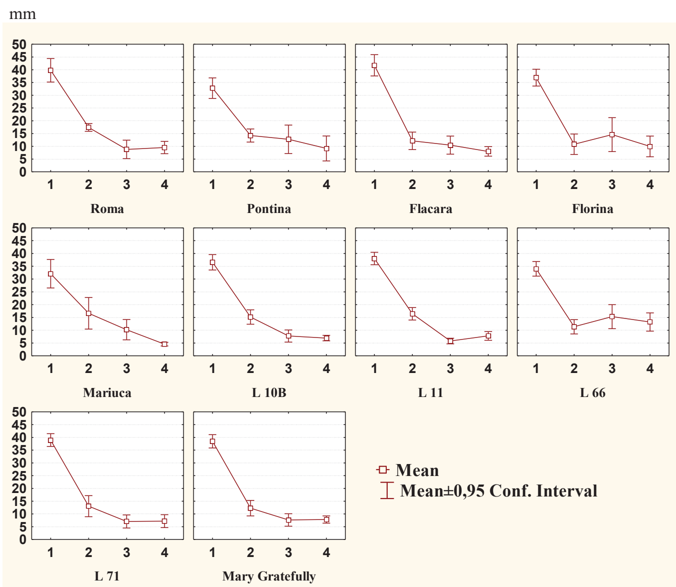

Figure 3. Influence of *F. oxysporum, F. solani, A. alternata* culture filtrates on stem growth in tomato seedlings
Vertical: stem length, mm
Horizontal: 1. H_2O (control); 2. *F. oxysporum*; 3. *F. solani*; 4. *A. alternata*

Based on the analysis of distribution dendrograms of varieties and lines of tomatoes, we found similarities and differences of distributions regarding the reaction of the embryonic root, stem and germination to the fungal metabolites (Figure 4). The highest similarity in the case of the embryonic root was found for L 10 B and L 11, of the stem - L 71 and Mary Gratefully, and of the germination – Pontina and L 66, Flacăra and L 11, which formed the smallest clusters. The other genotypes differed both from the control and from each other.

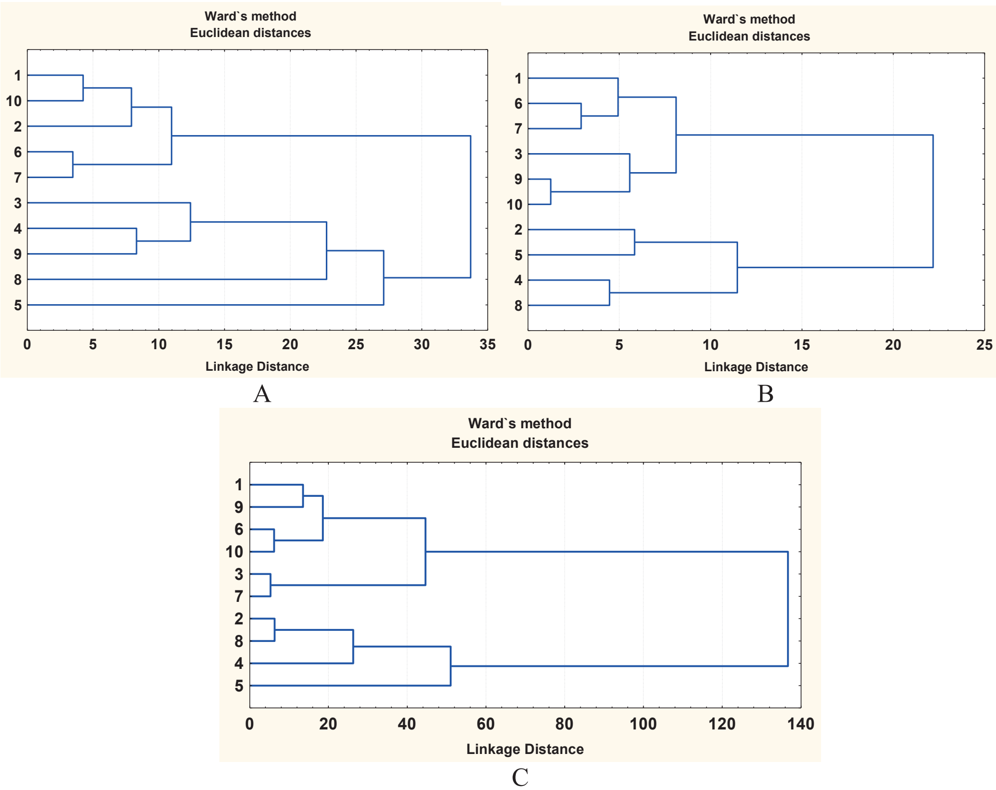

Figure 4. Dendogram of tomato varieties and lines distribution based on the reaction of the embryonic root (A), the stem (B) and the germination (C) to the fungi pathogens *F. oxysporum, F. solani, A. alternata*
1. Roma; 2. Pontina; 3. Flacara; 4. Florina; 5. Mariuca; 6. L 10 B; 7. L 11;
8. L 66; 9. L 71; 10. Mary Gratefully

Cluster analysis by *k*-means method showed that for all 3 studied characters, in the control variant the interclusterian variance was much higher than the intraclusterian one, which denotes that the 10 genotypes studied showed pronounced differences (Table 1).

Of great importance for the successful separation in clusters is the ability to discriminate characters and factors taken as cases - germination, root length, stem length. We can see that in general, the interclusterian and intraclusterian variance were much higher for germination, then for root length and lastly – for stem length.

In the case of germination, the separation of genotypes into clusters was successful for the all 4 variants - control, *F. oxysporum* CF, *F. solani* CF and *A. alternata* CF. It was observed that *A. alternata* CF was a factor with low genotypic differentiation power based on root length, and *F. oxysporum* CF - based on stem length, which indicates that the respective characters of the tomato genotypes studied did not specifically interact with these pathogens.

Table 1. Analysis of the inter- and intraclusterian variance in the interaction of tomato genotypes with some fungal pathogens

Variant	Interclusterian variance	Df	Intraclusterian variance	df	F	p
Germination						
Control (H_2O)	1079.601	2	180.468	7	20.94	0.00
F. oxysporum CF	1555.205	2	435.800	7	12.49	0.01
F. solani CF	761.029	2	517.560	7	5.15	0.04
A. alternata CF	1575.296	2	381.188	7	14.46	0.00
Root length						
Control (H_2O)	267.589	2	202.735	7	4.62	0.05
F. oxysporum CF	176.494	2	111.702	7	5.53	0.04
F. solani CF	160.621	2	31.820	7	17.67	0.00
A. alternata CF	85.309	2	96.880	7	3.08	0.11
Stem length						
Control (H_2O)	68.203	2	20.398	7	11.702	0.01
F. oxysporum CF	21.757	2	28.088	7	2.71	0.13
F. solani CF	79.243	2	16.118	7	17.207	0.00
A. alternata CF	26.683	2	19.773	7	4.72	0.05

By classifying the genotypes based on the 3 characters, it was found that in the control variant, cluster 1 had 6 genotypes - Rome, Florina, L 10B, L11, L71 and Mary Gratefully, with the highest values of the analyzed characters: germination - 96,38 %; root length - 58.43 mm and stem length - 38.1 mm. In the variant with FC, 4 of the mentioned genotypes - Rome, L 10B, L71 and Mary Gratefully formed cluster 3, with the highest germination values - 85.59%, the root and stem length being practically equal in the 3 clusters, which indicates that germination, compared to other 2 characters, was a factor with higher discriminant capacity (Table 2).

Table 2. Descriptive analysis of clusters

Cluster	Character	Control		Cultural filtrate	
		x	Genotype	x	Genotype
1	Germination, %	96.38	1 - Roma, 4 - Florina, 6 - L 10B, 7 - L 11, 9 - L 71,10 - Mary Gratefully	60.88	4 - Florina, 5 - Măriuca, 8 - L 66
	Root length, mm	58.43		18.80	
	Stem length, mm	38.1		11.86	
2	Germination, %	60.0	5 - Măriuca	76.64	2 - Pontina, 3 - Flacăra, 7 - L 11
	Root length, mm	39.1		19.42	
	Stem length, mm	32.1		10.74	
3	Germination, %	85.60	2 - Pontina, 3 - Flacăra, 8 - L 66	85.59	1 - Roma, 6 - L 10B, 9 - L 71, 10 - Mary Gratefully
	Root length, mm	51.63		18.87	
	Stem length, mm	36.20		10.05	

The evaluation of the data obtained regarding the crop structure (Table 3) demonstrated essential differences both after the general harvest and the commodity harvest that depended on the genotype and the weather conditions.

The climatic conditions of the year 2019 were extremely unfavorable for the growth of the plants, which favored the development of the virotic diseases that led to a considerable decrease of the productivity and the quality of the fruits. According to the data obtained in 2018, the general harvest ranged from 22.0 t/ha (Luci) to 78.6 t/ha (Măriuca), and the freight harvest from 40.1 to 69.6 t/ha, respectively.

Increased productivity in 2018 was recorded in the varieties Mariuca, Mary Gratefully, Flacara, L 66, whose productivity was 78.6, 73.0, 63.4 t/ha, 61.4 t/ha, respectively.

Table 3. The influence of the year conditions on the productivity characteristics of tomatoes

Variete, line	Harvest, t/ha						Share of fruit freight, %	
	General		% in comparison with 2018	Freight		% in comparison with 2018		
	2018	2019		2018	2019		2018	2019
Mary Gratefully	73.0	31.5	-56.9	59.6	28.2	-52.7	81.6	89.5
Roma	39.7*	29.0	-27.0	33.1*	23.7*	-28.8	83.4	81.7
Luci	22.0*	25.3*	+15.0	16.4*	20.9*	+27.4	76.8	82.6
Alex	41.4*	24.6*	-40.6	21.4*	19.9*	-7.1	51.7	80.9
Pontina	55.7*	29,3	-47.4	35.1*	25.5*	-27.4	63.0	87.0
Flacara	63.4*	25.7*	-59.5	44.6*	19.2*	-57.0	70.3	74.7
Kristina	32.3*	26.5*	-18.0	28.3*	22.5*	-20.5	87.6	84.9
Mariuca	78.6*	21.9*	-72.1	54.6*	19.5*	-64.3	69.5	89.0
Florina	59.7*	24.4*	-59.1	35.7*	22.2*	-40.8	59.8	91.0
Darsirius	56.0*	23.6	-57.9	50.6*	20.6*	-59.3	90.4	87.3
L 10B	55.4*	28.7	-48.2	48.6*	23.4*	-51.9	87.7	81.5
L 11	59.4*	17.0*	-71.4	52.3*	14.7*	-71.9	88.0	86.5
L 66	61.4*	28.2	-54.1	54.3*	25.1*	-53.8	88.4	88.6
L 71	50.6*	32.2	-36.4	39.1*	27.7	-29.2	77.3	86.0

*veridical difference at the $p < 0.05$ level of the control.

The climatic conditions of the 2019 year have significantly influenced both the general and the freight harvest. Significant decrease of the general harvest compared to 2018 year was attested to all the evaluated forms, falling within the limits of 18.1 ... 71.4%, except for the variety Luci at which the general harvest in 2018 year was of 22.0 t/ha, and in 2019 year - 25.3 t/ha. The highest productivity in 2019 year was found in the varieties Mary Gratefully, Pontina, Roma and lines L 10, L 66, L 71. Commodity harvest compared to 2018 years decreased by 7.1… 71.9%. The share of fruit freight in the samples taken in the study proved to be quite high and ranged from 51.7% (Alex) to 90.4% (Darsirius) in 2018 year and from 74.7% (Flacara) to 91.0% (Florina) in 2019.

CONCLUSIONS

It was found that the reaction of tomato plants (germination, root and stem growth) to *F. oxysporum, F. solani* and *A. alternata* CF's under controlled conditions was different and depended on the genotype growth organ, and the fungal species.

Cluster analysis by the *k*-means method found that all fungal species showed a higher discriminative capacity of tomato clusters for seeds germination, and for the length of the stem and root - *F. oxysporum* and *F. solani*, respectively, which reveals the specificity of more pronounced interaction with these pathogens.

By *k*-mean cluster analysis it was found that in the variant with fungal culture filtrates, 4 of the tested genotypes - Rome, L 10B, L71 and Mary Gratefully formed the cluster with the highest values of germination, root and stem length, which denotes their less pronounced sensitivity.

The highest productivity in the 2019 year showed the Mary Gratefully (31.5 t/ha) Rome (30.3 t/ha), Pontina (29.3 t/ha) and L 71 lines (32.2 t/ha), L 10 B (28.7 t/ha), L 66 (28.2 t/ha) varieties. The Mary Gratefully variety and the L 71 line can be successfully used in breeding for creating of new varieties with high adaptability to the pedoclimatic conditions of the Republic of Moldova.

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