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INFLUENCE OF THE *ALTERNARIA* AND *FUSARIUM* SPP. CULTURE FILTRATES ON THE GROWTH OF TOMATO PLANTS IN EARLY ONTOGENESIS

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Abstract

In the paper are presented the peculiarities of reaction of the new created tomato lines to the treatment with the culture filtrates (CF) of some important phytopathogens - Alternaria alternata, Fusarium oxysporum, F. solani, based on germination, stem length and embryonic roots. The clusterian analysis (k-mean method) proved that, in comparison to A. alternata, the F. oxysporum and F. solani fungus species exhibited a higher discriminative capacity of the tomato clusters based on the root and stem length, which reveals the specificity of greater interaction with these pathogens. Clusters of tomato genotypes with diminished reaction to Fusarium spp., A. alternata pathogens have been identified, which is important for their involvement in the breeding programs. By factorial analysis of the variance, it was found that for the embryonic root of the tomatoes, the major contribution to the source of variation in character had the genotype of the plant, its contribution constituting 46.7%, and for the growth of the stem – factor of isolate: 62.5%.

Key words: *tomatoes, resistance, fungal pathogens, Fusarium spp., A. alternate*.

INTRODUCTION

The growth and development of this culture are affected by the strong influence of mycotic diseases and low temperatures at early stages of development (Foolad, 2007), thus demonstrating reduced genetic resistance to the mentioned factors. Among the biotic factors unfavorable to the growth and development of tomato plants under the conditions of the Republic of Moldova lately, there is noticed the root fusariosis, the main causative agent being *Fusarium oxysporum var. orthocerus* spp., which causes root rot at various stages of development, petiole and leaf staining, weakening and wilting of plants (Lupascu, Rotaru & Mihnea, 2009), and alternariosis (*Alternaria alternata*) – brown spot of leaves, shoots and fruits (Lupascu et al., 2013; Mamgain A., Roychowdhury, Tah, 2013).

The priority direction in the strategy of plant improvement at the stage of adaptive intensification of agriculture is combining the resistance of varieties and hybrids to environmental stressors, including diseases, with the high level of harvest and quality of production (Foolad, 2007; Barone et al., 2008; Lupascu, 2016).

Creating resistant tomato varieties is one of the most effective strategies for controlling of alternariose (Foolad et al. 2002; Zhang et al., 2003; Matharu, Sharma, Manrao, 2006; Çalış, Topkaya, 2011; Mihnea, Lupascu, Gavzer, 2018). Regarding the need to create sustainable resistance sources, special attention is paid to the interactions of tomato plants with pathogens and environmental conditions (Lupascu et al., 2013).

Various relationships are established between plant and pathogen, defined by genotype resistance, fungal virulence, environmental conditions, and so on (Lupascu et al., 2015; Lupascu 2016). The creation of new plant genotypes, tolerant or resistant to pathogens, stressing temperatures, through genetic amelioration and / or transformation, is an effective way of protecting plants against adverse environmental conditions.

The aim of the research was to identify tomato perspective lines with complex resistance to *Alternaria alternata* and *Fusarium spp*., based on the reaction to pathogen culture filtrates.

MATERIALS AND METHODS

As a research material, 9 tomato perspective lines and control cultivar 'Mary Gratefully', which show a complex of valuable characters (created in the Institute of Genetics, Plant Physiology and Plant Protection of the Republic of Moldova), served as a research material.

The culture filtrates (CF) of *F. oxysporum*, *F. solani*, and *Alternaria alternata* (isolated from tomato ill plants) prepared by inoculating the mycelium in the Czapek-Dox liquid medium and subsequently growing at 22- 24ºC for 21 days.

Tomato seeds were treated with CF of fungi for 18 hours. As a control served the seeds kept in the distilled water.

Cultivation of the seedlings took place in Petri dishes on filter paper wetted with distilled water at a temperature of 22-24°C for 6 days. As test-index of plant reaction served the important early growth and developmental characters of tomato ontogenesis – germination, roots length and stem length.

Clusterian analyzes were performed by dendrogram construction method (Ward method) and *k*-means method (Savary, 2010).

To assess the role of genotype, fungal species and their interaction in the source of variation of the quantitative characters, the bifactorial analysis of the variance ANOVA was applied.

RESULTS AND DISCUSSIONS

Testing the reaction of tomato plants to the seeds treatment with culture filtrate of *A. alternata, F. oxysporum, F. solani*, showed that under the action of pathogen metabolites, seeds germination, growth and development of the embryonic root and strain were suppressed in most of the cases. The plant response was influenced by genotype, analyzed character and fungal species, being largely determined by the organ under test.

For example, with regard to seeds germination capacity of the perspective lines, after their treatment with mentioned CF fungi have been found a differentiated reaction (Figure 1), however in most cases not significantly affected.

It should be mentioned that *A. alternata* CF in 4 cases out of 10 stimulated seeds germination by 2-9.5%, and *F. oxysporum* and *F. solani* – by 6.0-7.0% for Mary Gratefully and 11.0 - 17.4% – to L 304 varieties, respectively. Under the influence of *A. alternata* CF inhibition was -3.0 ... -8.0%. Significant repression was seen in L 303 (-23.0%), L 305 (- 15%), L 309 (-16.0%) under the influence of *F. oxysporum* CF and L 310 (-15.0%) under the influence of *F. solani* CF.

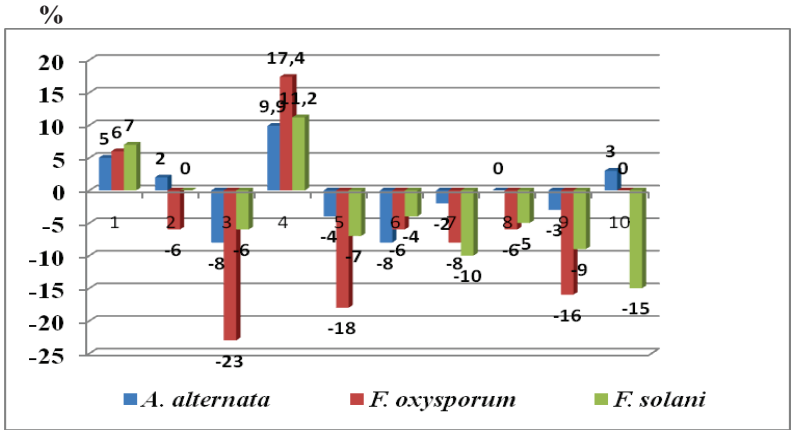


Fig. 1. Influence of fungus culture filtrates on the seeds germination of the tomato perspective lines

1 – Mary Gratefully, 2 – L 302, 3 – L 303, 4 – L 304, 5 – L 305, 6 – L 306, 7 – L 307, 8 – L 308, 9 – L 309, 10 – L 310

In the case of root, the genotypes also showed quite differentiated sensitivity to fungus CF (Table 1). Thus, the above-mentioned culture filtrates stimulated the growth of the root in the Mary Gratefully and L 304 cultivars. The evaluated lines were most strongly influenced by *F. oxysporum*, averaged over the control ranging from -3.5 ... -33.0 %. There were strong inhibitions at L 305, L 307, L309. In the case of *F. solani* CF in 7 cases out of 10, there was confirmed stimulation of embryonic root growth and in 2 cases: L 309, L 310 – strong suppression. Only at genotype L 303 inhibition was insignificant: -1.5%. It should be noted that *A. alternata* in 8 cases out of 10 influenced by stimulating (+2.0 ... + 10.0%). The strong sensitivity was demonstrated by L 310 (-21.2%) and insignificant by L 302 (-4.8%). So the lines have been strongly distinguished on the basis of the analyzed character, which reveals the opportunity to identify the most resistant genotypes.

In the case of strain length, a higher amplitude of variability was identified in response to fungi CF. The inhibition of the strain compared to the control ranged from -19.9 ... -48.9% to *F. oxysporum*, -7.2 ... -54.3% – *F. solans*, -3.4 ... -30.6% – *A. alternata*. For example, in the variant with *F. oxysporum* CF there was noted strong character inhibition at L 307, L 308, L 309; L 305 – by 48.9, 33.7, 33.5 and 30.2% and insignificant stimulation at L 310 and L 304 – by 12.2 and 5.1%, respectively.

The data show a higher sensitivity of the strain to fungal pathogens compared to germination and root length.

According to the dendrograms (Fig. 1) analysis of the tomato lines distribution based on the reaction to the 3 CF, similarities and differences were found regarding the reaction of the embryonic root and strain to the fungal metabolites. The highest similarity was found for L 306 and L 308 that formed a small cluster, followed in ascending order by L 302 and L 304, L 305 and L 307. The other clusters distinguished themselves both as control and between them.

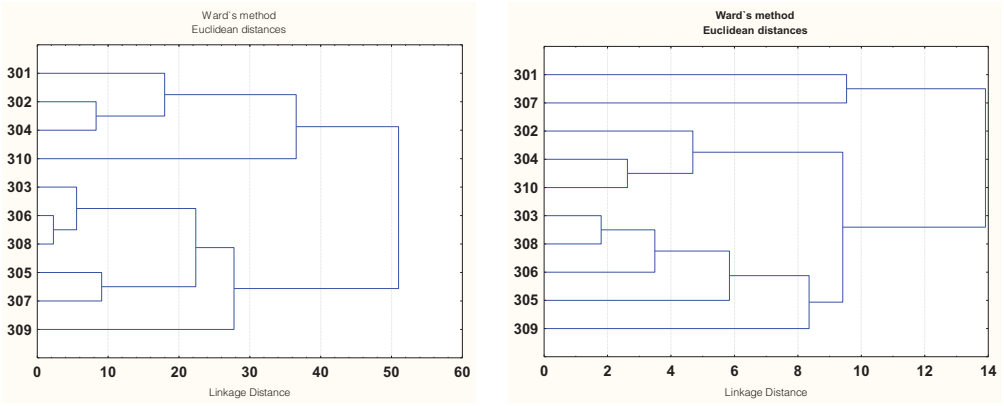
In terms of strain length, L 303 and L 308 formed a small cluster, which revealed their high similarity in reaction to fungal metabolites.

Table 1. Influence of *Alternaria alternata* and *Fusarium spp*. on some growth and development characters in tomatoes

Nr.	Variant	Germination, %	% to control	Root length, mm	% to control	Stem length, mm	% to control
1	2	3	4	5	6	7	8
Mary Gratefully							
1	H_2O (control)	92.5	100	44.4±1.34	100	21.6±0.66	100
2	FC *A. alternata*	96.7	104.5	45.3±1.34	102.0	15.0±0.66*	69.4
3	FC *F. oxysporum*	98.0	105.9	50.6±1.44	114.0	17.3±0.69*	80.1
4	FC *F. solani*	99.2	107.2	65.6±1.63*	147.7	17.4±0.63*	80.6
L 302							
1	H_2O (control)	72.5	100	41.8±1.66	100	15.5±0.83	100
2	*A. alternata* CF	74.2	102.3	39.8±1.68	95.2	12.2±0.83	69.4
3	*F. oxysporum* CF	68.3	94.2	44.6±2.0	106.7	14.3±0.85	80.1
4	*F. solani* CF	72.5	100	52.8±2.26*	126.3	14.3±0.88	80.6
L 303							
1	H_2O (control)	75.0	100	39.9±1.68	100	16.6±0.75	100
2	*A. alternata* CF	69.2	92.3	41.5±2.18	104.0	15.7±0.95	94.6
3	*F. oxysporum* CF	57.7	76.9	37.5±2.06	94.0	11.9±0.73*	71.7
4	*F. solani* CF	70.8	93.7	39.3±2.05	98.5	12.4±0.90*	74.7
L 304							
1	H_2O (control)	66.7	100	42.8±1.96	100	13.7±0.74	100
2	*A. alternata* CF	73.3	109.9	46.8±2.17	109.3	15.5±0.86	113.1
3	*F. oxysporum* CF	78.3	117.4	48.1±1.91	112.4	14.4±0.90	105.1
4	*F. solani* CF	74.2	111.2	50.2±2.45*	117.3	12.8±0.87*	92.1
L 305							
1	H_2O (control)	77.5	100	41.3±1.97	100	13.9±0.88	100
2	*A. alternata* CF	74.2	95.7	45.8±2.06	110.9	12.7±0.76	91.4

3	*F. oxysporum* CF	63.3	81.7	28.8±1.94*	69.7	9.7±0.60*	69.8
4	*F. solani* CF	72.5	93.5	45.8±2.04	111.0	15.5±0.89	111.5
				L 306			
1	H_2O (control)	93.3	100	41.8±1.46	100	16.6±0.69	100
2	*A. alternata* CF	85.8	92.0	44.6±1.48	106.7	16.0±0.77	96.4
3	*F. oxysporum* CF	88.2	94.5	38.8±1.74	92.8	11.7±0.58*	70.5
4	*F. solani* CF	89.2	95.6	42.3±1.42	101.2	15.4±0.80*	92.8
				L 307			
1	H_2O (control)	95.8	100	47.0±1.44	100	17.8±0.77	100
2	*A. alternata* CF	94.2	98.3	49.0±1.57	104.3	17.2±0.82	96.6
3	*F. oxysporum* CF	88.3	92.2	31.5±1.19*	67.0	9.1±0.41*	51.1
4	*F. solani* CF	93.3	97.4	51.6±1.34	109.8	19.5±0.78	109.6
				L 308			
1	H_2O (control)	91.7	100	39.6±1.35	100	16.3±0.53	100
2	*A. alternata* CF	91.7	100	44.8±1.37	109.8	14.4±0.80	88.3
3	*F. oxysporum* CF	86.5	94.3	38.2±1.38	96.5	10.8±0.42*	66.3
4	*F. solani* CF	87.5	95.4	42.4±1.42	107.1	12.9±0.65*	79.1
				L 309			
1	H_2O (control)	76.7	100	46.7±1.82	100	18.8±0.71	100
2	*A. alternata* CF	74.2	96.7	47.7±1.84	102.1	15.7±0.87	83.5
3	*F. oxysporum* CF	64.2	83.7	36.1±2.04*	77.3	12.5±0.80*	66.5
4	*F. solani* CF	62.5	81.5	25.3±1.74*	54.2	8.6±0.72*	45.7
				L 310			
1	H_2O (control)	84.2	100	59.4±1.91	100	14.7±0.70	100
2	*A. alternata* CF	86.7	103	46.8±2.00*	78.8	14.8±0.84	101.0
3	*F. oxysporum* CF	84.2	100.0	58.7±2.20	98.8	16.5±0.79	112.2
4	*F. solani* CF	71.7	85.1	36.6±1.96*	38.4	11.8±0.81*	80.3

*- authentic control distinction ($p \leq 0,05$).



A B

Fig. 1. The distribution of tomato perspectives lines in the response of embryonic root (A) and strain (B) to fungal pathogens *A. alternata* and *Fusarium* spp.
1 – Mary Gratefully, 2 – L 302, 3- L 303, 4 – L 304, 5 – L 305, 6 – L 306, 7 – L 307, 8 – L 308, 9 – L 309, 10 – L 310

The clusterian analysis (*k*-means method) demonstrated that the *F. oxysporum* and *F. solani* fungal species showed a higher discriminative capacity for tomato clusters for root and stem lengths compared to *A. alternata*, which reveals the specificity of greater interaction with these pathogens (Figure 2).

By *k*-means analysis of 3 clusters programmed according to the possible values of the analyzed parameters – big, medium, small, it was determined that for the length of the roots as members of cluster 1 were: L 301, L 302, L 304, L 310; cluster 2: L 303, L 306, L 308, L 309; cluster 3: L 305, L 307.

For strain length, as members of cluster 1 were: L 303, L 308, L 309; cluster 2: L 301, L 302, L 304, L 310; cluster 3: L 305, L 306, L 307.
Clusters of tomato lines with weak reaction to the Fusarium spp., A. alternata pathogens have been identified, which is important for their effective involvement in the breeding programs.

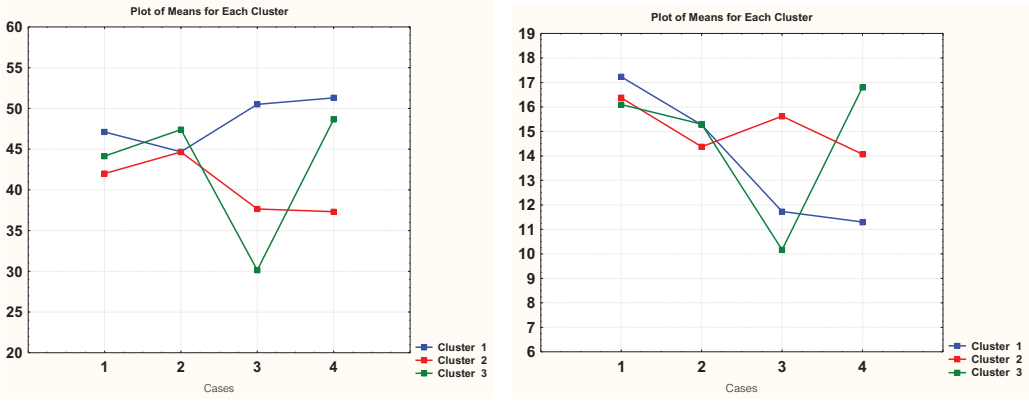


Fig. 2. Clusterian analysis (*k* - means) of tomato genotypes based on the reaction of the embryonic root (A) and stem (B) to the fungal metabolites
Horizontal: 1 – H_2O, 2 – *A. alternata*, 3 – *F. oxysporum*, 4 – *F. solani.*
Vertically: 1, 2, 3 – clusters of genotypes.

By the factorial analysis of the variance (Table 2) it was found that for the embryonic root of tomatoes, the major contribution to the source of variation had the genotype of the plant, its contribution constituted 46.7%, and for the isolated isolate strain: 62.5%. It should be noted that an imported role has held *isolate x genotype* interactions. Their factorial weight was for the embryonic root of 31.6%, and for the stem – 14.3%. So the embryonic root depends more on the particular way of interacting with the species researched by the fungus.

Table 2. Factorial analysis of tomato genotype x fungal pathogen relationships

Source of variation	Degree of freedom	Average square sum	Contribution to source of variation,%
Length of the root			
Genotype	9	7736*	46.7
Isolate	3	3288*	19.9
Genotype x isolate	27	5238*	31.6
Random effects		294	1.8
Length of the stem			
Genotype	9	821.6*	21.7
Isolate	3	2369.6*	62.5
Genotype x isolate	27	542.7*	14.3
Random effects	579	55.6	1.5

*- $p \leq 0,05$.

CONCLUSIONS

As a result of the analysis of tomato perspectives (identified in competition tests) in *Alternaria alternata* and *Fusarium* spp., It was found that in most cases they did not significantly affect the germination of the seeds. Significant inhibition was seen in L 303 (-23.0%), L 305 (- 15%), L 309 (-16.0%) lines under the influence of *F. oxysporum* CF and L 310 (-15.0%) under the influence of *F. solani* CF.

The growth of the root at the evaluated lines was most strongly influenced by *F. oxysporum*, averaged over the control range ranging from -3.5 ... -33.0%. Regarding the strain, a greater magnitude of variability was identified in response to CF of the fungus.

Clusterian analysis (k-media method) demonstrated that the *F. oxysporum* and *F. solani* fungal species demonstrated a higher discriminative capacity of tomato clusters for the length of roots and stems compared to *A. alternata*, which reveals the specificity of more intense interaction with these pathogens.

Clusters of tomato genotypes with diminished reaction to *Fusarium spp*., *A. alternata* pathogens have been identified, which is important for their involvement in the breeding programs in order to create resistant descendants.

By bifactorial analysis of the variance, it was found that for the embryonic root of tomatoes, the major contribution to the source of variation had the genotype of the plant, its contribution constituting 46.7%, and for the isolated isolate strain: 62.5%. An important role had isolate x genotype interactions, their factorial weight being 31.6% for embryonic root and 14.3% for the stem. So the embryonic root reacts more specifically with the A. alternata, F. oxysporum, F. solani fungus species.

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