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THE INFLUENCE OF *FUSARIUM OXYSPORUM* AND *ALTERNARIA ALTERNATA* FUNGI ON VARIABILITY AND HERITABILITY OF THE TOMATO GROWTH CHARACTERISTICS

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Abstract

As a result of the analysis of the sensitivity of the tomato perspective lines to the culture filtrates (CF) Alternaria alternata and Fusarium oxysporum, it was found that in the most of the cases they did not significantly influence seeds germination, but in all the cases, inhibition of the embryonic root length and stem occurred. By bifactorial analysis of the variance it was found that for the seeds germination, the genotypic factor was the most important in the reaction to F. oxysporum and A. alternata isolates, and for the growth of embryonic root and stem in both variants a major influence belongs to the fungus isolate. Genotypic and phenotypic variations varied to a large extent depending on the isolate and the analyzed character. High coefficient of heritability in the broad sense (h^2 = 0.60-0.95) indicates a good heredity of the studied characters in the interaction with the isolates of F. oxysporum and A. alternata fungi. We mention that the coefficient of genotypic variation varied widely – 17.8-73.1% for the studied characters, which proves the genetic and environmental nature of their variability.

Key words*: tomatoes, variability, growth organs, F. oxysporum, A. alternata.*

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.), an important widespread crop, is some of the most widely consumed and popular vegetables, widely grown worldwide and particularly profitable for growers (Roiola et al., 2014; Nasir et al., 2015).

Globally, tomatoes are an important part of a diverse and balanced diet (Willcox et al., 2003), providing a wide variety of nutrients (Ilahy et al., 2016), vitamins, carotenoids and phenolic compounds (Raiola et al., 2014; Martí et al., 2016; Li et al., 2018).

These bioactive compounds have a wide range of physiological properties, including antiinflammatory, antiallergenic, antimicrobial, vasodilating, antithrombotic, cardio-protective and antioxidant effects (Raiola et al, 2014). Also, fresh and processed tomatoes are the richest sources of antioxidant lycopene in the human diet (Viuda-Martos et al., 2014; Nasir et al., 2015), which protects the human body from free radicals and reduces the risk of cancer (Giovannucci, 1999).

Carotenoids and polyphenolic compounds contribute to the nutritional value of tomatoes and improve their functional attributes and sensory qualities, including taste, aroma and texture (Raiola et al., 2014; Tohge & Fernie, 2015; Martí et al., 2016).

Cultivated tomatoes have a limited genetic diversity due to their long selection and oriented towards certain traits during evolution and domestication (Bai & Lindhout, 2007; Blanca et al., 2015).

Tomato resistance to disease is often associated with a number of undesirable traits, which is why resistance donors are not acceptable in practice (Chaerani & Voorrips, 2006).

For these reasons, tomatoes are prone to a high incidence of disease, during the cultivation and post-harvest period being affected by over 200 diseases caused by various pathogens (King & Lively, 2012; Singh et al., 2017).

Recently, tomatoes have a high frequency of fungal pathogens of *Fusarium* spp. causing root rot at various stages of development, yellowing of lower leaves, vascular necrosis, wilting of plants, defoliation, and eventually plant death (Szczechura et al., 2013; McGovern, 2015; Bodah, 2017; Srinivas et al., 2019) and *Alternaria* spp. which is manifested by brown spots on leaves, shoots, fruits (Mamgain et al.,

2013; Lupascu et al., 2015; Mihnea et al., 2018).

As alternariosis develops, defoliation occurs, starting with the older leaves and moving toward the younger ones, and necrotic lesions can be seen on flowers and stems (Strandberg, 1992). The disease can lead to complete defoliation, strongly influencing the photosynthetic efficiency of the plant, productivity and fruit quality (Rotem, 1994; Lawrence et al., 2000).

For the successful management of these diseases, it is necessary to use varieties with high genetic performance, a factor that is a decisive link of the innovative progress in agriculture and ensures the achievement of high quantities of high quality production, with required organoleptic properties (Carli et al., 2011; Ercolano et al., 2008; Mihnea et al., 2016; Seymour et al., 2002). Plantation rotation, fungicide diversification (Olaya et al., 2017; Malandrakis et al., 2018), the use of different agronomic methods, starting from the use of healthy seeds or seedlings, soil moisture control, removal of infected plants, plant debris and weeds, increasing plant vigor through proper fertilization management are decisive factors for achieving the desired goals (Foolad et al., 2008; Small et al., 2015; McGovern, 2015; Gamliel et al., 2009). Obtaining stable crops for tomatoes, reducing losses due to diseases and unfavorable environmental factors can be achieved by creating resistant varieties with ecological stability and high plasticity (Mihnea et al. 2016; Mihnea, 2017).

The aim of our research was to determine the influence of *F. oxysporum* and *A. alternata* fungi on the organs of growth and development of tomato plants in the early stage of ontogenesis, as well as on their variability and heredity.

MATERIALS AND METHODS

The experiments were carried out in laboratory conditions at the Laboratory of Applied Genetics, Institute of Genetics, Physiology and Plant Protection.

Mary Gratefully and the lines L 302, L 303, L 304, L 305, L 306, L 307, with agronomically valuable characteristics, obtained from the following hybrid combinations: F_{14} Potoc x F_1 (*Gruntovschi gribovschi x L. chilense*), F_7 (Maestro x Irisca), F_{12} (Novicioc x Iuliana), F_7 (Maestro x Irișca) F_8 (Mihaela x Dwarf Moneymaker), F_7 (Maestro x Dicaia roza), correspondingly, were used as material for the assessment of resistance to fungal diseases. Mary Gratefully approved variety was used as a control.

To establish pathogenic agents that cause root rot and brown leaf spot in tomatoes, they were initially isolated in aseptic conditions on must-agar and PDA (Potatoes Dextrosis Agar) media according to classical methods (Bilay, 1982). These media are best optimized for the isolation, cultivation and study of the morphologic-cultural characteristics of pathogens. Small fragments of tissue from the base of the stem, leaf, petiole of tomato plants were used. The fragments were sterilized in a 2% solution of calcium hypochloride for 1-2 min, then washed 2-3 times in bidistilled water, squeezed between 2 sheets of filter paper, and placed on a medium near the gas flame.

The species of causative agents were identified based on macro- and microscopic characteristics, according to mycological identification guides (Bilay, 1977; Barnett & Hunter, 1998; Ellis & Ellis, 1985).

Culture filtrates (CF) of 3 isolates of *F. oxysporum* and *A. alternata* fungi (CF1, CF2, CF3), isolated from tomato plants with signs of disease, were used.

CF were prepared by inoculating the mycelium in Czapek-Dox (Tuite, 1969) liquid medium and subsequently culturing at 22-24°C for 21 days.

Tomato seeds were treated with fungal CF for 16 hours. The seeds kept in distilled water served as control. The seedlings were grown out in three replications in Petri dishes on filter paper moistened with distilled water at a temperature of 24-25°C for 6 days. As a test index of plant reaction, served important growth and development characteristics of tomatoes in the early stage of ontogenesis – germination, root length and stem length.

The share of phytopathosystem components was determined by bifactorial analysis of the variance.

For the analysis of genetic variability, heritability and genetic progress, the following formulas were applied:

$\sigma^2_g = (MSS - MSE)/r;$

$\sigma^2_{ph} = \sigma^2_e + \sigma^2_G;$

$h^2 = \sigma^2_g / \sigma^2_{ph}$ x 100%;
$PCV = 100 \times \sqrt{\sigma^2_{ph}} / X$;
$GCV = 100 \times \sqrt{\sigma^2_g} / X$;
$GA = K \times (\sigma P) \times h^2$;
$GA,\% = 100 \times K \times h^2 \times \sigma_{ph} / X$, in which:

σ^2_g – genotypic variance; σ^2_{ph} – phenotypic variance; σ^2_e (error variance, or VE) = MSE; h^2 – coefficient of heritability in the broad sense; PCV – phenotypic coefficient of variation; GCV – coefficient of genotypic variation; X – general average of the character; GA – genetic advantage; K – selection differential = 2.06 at the selection pressure of 5%; σ_{ph} – general standard deviation of character (Rameeh, 2014; Adeniji, 2018; Balkan, 2018).

Cluster analysis was performed by the *k*-means method (Savary et al., 2010), programming 3 clusters according to the possible values of the characters: small, medium and high.
The data obtained were statistically processed in the software package STATISTICA 7.

RESULTS AND DISCUSSIONS

Testing of the reaction of tomato plants to the treatment of CF seeds of 3 isolates of the fungus *F. oxysporum* showed that under the action of pathogen metabolites in most lines there was an inhibition of germination, embryonic root length and stem. In this case, the reaction of the plants depended on the genotype, the factor analyzed and isolated.

Seed germination analysis showed that CF in 14 cases out of 28 produced inhibition by 2.3 - 9.3%, in 3 – 11.0-14.0% and in 2 cases more than 20%. A differentiated reaction of tomato genotypes was noted. In the Mary Gratefully variety under the influence of *F. oxysporum* CF 2, germination was inhibited by 14.0%, and in the lines L 302 and L 306 – stimulated by 2.7% and 16.7%, respectively. An inhibition was also observed with 21.0% in the Mary Gratefully variety under the influence of CF3 and with 29.7% in L 306 – under the influence of CF1 (Figure 1 A). The genotypes L 303, L 304 and L 307 showed resistance to all three isolates.
Regarding the *A. alternata* fungus, it was found that when treating the seeds with CF, under the action of the pathogen metabolites in most lines there was an insignificant repression of seed germination. *A. alternata* CF inhibited seed germination by 1.0… 11.8%. Insignificant stimulation was recorded at L 302 (1.3%) and L 303 (1.2%) (Figure 1 B). The L 303 and L 307 lines showed an increased resistance to all 3 isolates, which can be used in the improvement process as the most resistant.
Genotypes L 303 and L 307 have shown complex resistance and are of interest in breeding tomatoes as a source of resistance to *F. oxysporum* and *A. alternata*.

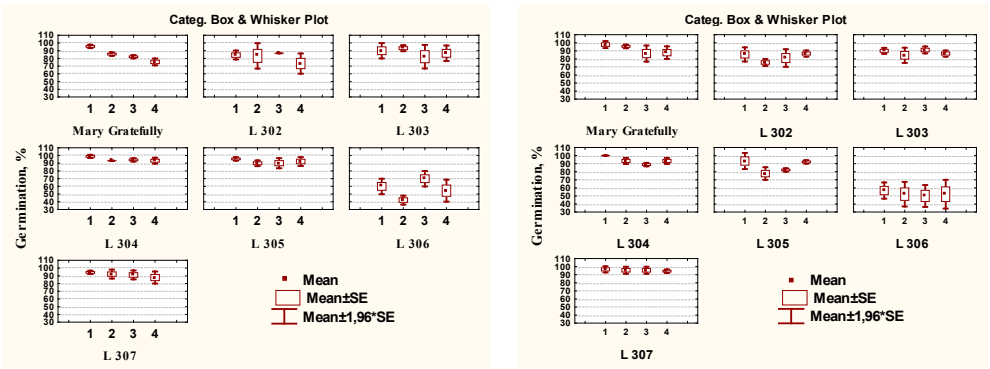
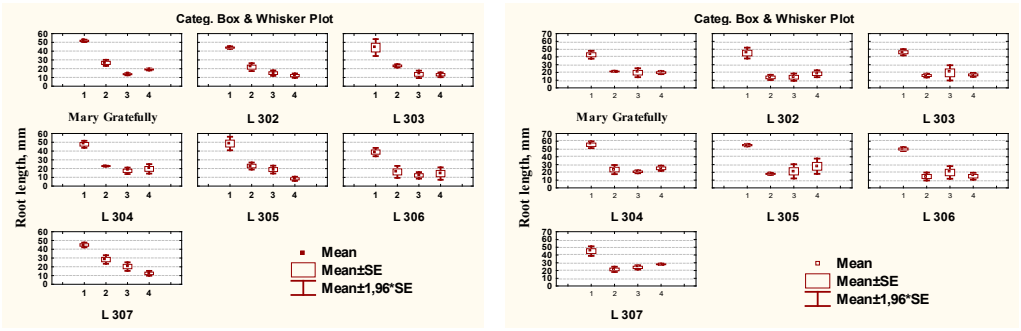


Figure 1. Influence of *F. oxysporum* (A) and *A. alternata* (B) culture filtrates on seeds germination (%) on the Mary Gratefully and some tomato lines
Horizontal: 1 – H_2O (control), 2 – CF 1, 3 – CF 2, 4 – CF 3

Regarding root growth, it was found that *F. oxysporum* and *A. alternata* CF had a different effect (Figure 2). Thus, *F. oxysporum* CF1 inhibited root length by 36.4-53.0% in the genotypes under study. In the case of CF2 and CF3, there was a much stronger inhibition: 56.6-78.3% and 61.1-82.9% of the control, respectively.

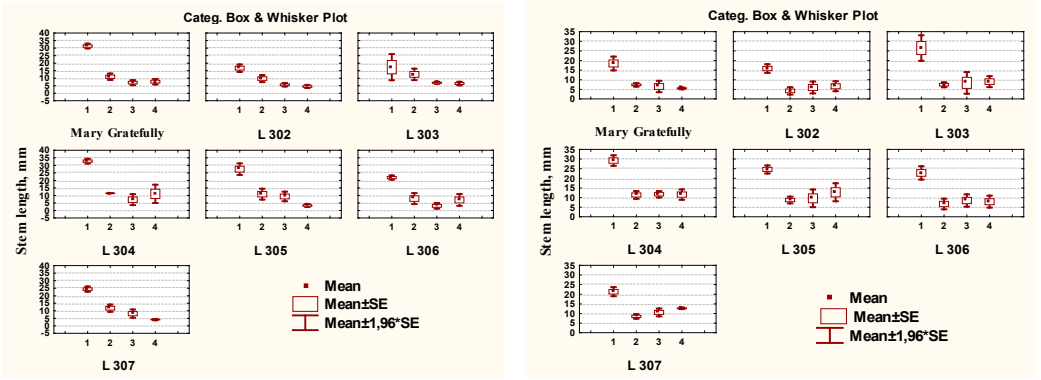
Genotypes were found to be quite sensitive to *A. alternata* CF. Thus, *A. alternata* CF inhibited root growth within 38.2-69.6%. The evaluated genotypes were the most strongly influenced by CF1 and CF2, the average values in relation to the control varying in the limits of 50.8-69.6% and 46.4-67.3%, respectively. There were strong inhibition at L 302, L 305, L 306. The lowest sensitivity of the embryonic root in the studied CF was recorded at L 307.



A B

Figure 2. Influence of *F. oxysporum* (A) and *A. alternata* (B) culture filtrates on root growth in tomato seedlings

In the case of the reaction of the strain to *F. oxysporum* CF, the inhibition in relation to the control was 34.6-88.3: CF1 – 34.6-66.0%, CF2 – 63.9-84.9%, CF3 – 64.9-88.3%, and for *A. alternata* CF – 40.7-72.6%: 40.7-72.6% for CF1, 49.5-70.7% – CF2 and 40.7-70.1% – CF3 (Figure 3). So, as in the case of the root, the stem was the most strongly affected by CF2 and CF3.



A B

Figure 3. Influence of *F. oxysporum* (A) and *A. alternata* (B) culture filtrates on stem growth in tomato seedlings

Cluster analysis (*k*-means method) showed that for all 3 studied characters, in the control variants and with *F. oxysporum* and *A. alternata* CF's, the intercluster variant was much higher than the intracluster one, which indicates that the 7 genotypes taken into account showed distinct pronounced differences. The only exception was *F. oxysporum* CF1 for root and stem length, in which the intercluster variance was lower than the intracluster variance, which indicates the poor specificity of the genotype reaction to this isolate (Tables 1, 2).

Table 1. Analysis of inter- and intraclusterian variance in the interaction of tomato genotypes with *F. oxysporum*

Variant	Interclusterian variance	df	Intraclusterian variance	df	F	p
Germination						
Control (H_2O)	967.924	2	102.890	4	18.81	0.01
FC1	2029.844	2	13.625	4	297.96	0.00
FC2	303.562	2	90.213	4	6.73	0.05
FC3	1116.617	2	33.880	4	65.92	0.00
Length of the root						
Control (H_2O)	67.902	2	28.940	4	4.69	0.09
FC1	36.204	2	44.485	4	1.63	0.30
FC2	36.640	2	12.497	4	5.86	0.06
FC*3*	73.239	2	12.250	4	11.96	0.02
Length of the stem						
Control (H_2O)	203.641	2	18.597	4	21.90	0.01
FC1	1.841	2	9.457	4	0.39	0.70
FC2	10.113	2	7.527	4	2.69	0.18
FC*3*	27.041	2	6.457	4	8.38	0.04

df= Freedom degree; F= Fisher's criterion; p= error

Table 2. Analysis of inter- and intraclusterian variance in the interaction of tomato genotypes with *A. alternata*

Variant	Interclusterian variance	df	Intraclusterian variance	df	F	p
Germination						
Control (H_2O)	12297.661	2	28.093	4	92.38	0.000
FC1	1402.510	2	46.0667	4	60.89	0.001
FC2	1257.202	2	102.087	4	24.63	0.006
FC3	1252.964	2	45.173	4	55.47	0.001
Length of the root						
Control (H_2O)	137.843	2	19.072	4	14.46	0.016
FC1	62.877	2	20.072	4	6.27	0.059
FC2	33.626	2	21.732	4	3.09	0.154
FC3	118.277	2	39.840	4	5.94	0.063
Length of the stem						
Control (H_2O)	76.240	2	26.617	4	5.73	0.067
FC1	15.528	2	9.547	4	3.25	0.145
FC2	23.808	2	2.032	4	23.44	0.006
FC3	45.364	2	2.125	4	42,70	0,002

df= Freedom degree; F= Fisher's criterion; p= error

By classification, based on the three characters and descriptive analysis of the clusters, have been identified genotypes of tomatoes that were located in cluster 3, with complex resistance to both pathogens – L 304, L 307; resistance to *F. oxysporum* – L 303, L 305. Germination, compared to other 2 characters, was a factor with higher discriminant capacity (Table 3).

Table 3. Descriptive analysis of clusters

Cluster	Character	*F. oxysporum*		*A.alternata*	
		x	Genotype	x	Genotype
1	Germination, %	55.5	L 306	51.5	L 306
	Root length, mm	14.2		16.3	
	Stem length, mm	6.0		7.7	
2	Germination, %	81.1	Mary Gratefully, L 302	85.7	Mary Gratefully, L 302, L 303, L 305
	Root length, mm	18.1		19.6	
	Stem length, mm	7.8		7.7	
3	Germination, %	90.5	L 303, L 304, L 305, L 307	93.5	L 304, L307
	Root length, mm	18.4		23.9	
	Stem length, mm	9.1		11.1	

The processing of experimental data by bifactorial analysis of the variance allowed to assess the variability and degree of influence of the isolate, genotype and their interaction in the share of the phenotypic manifestation of growth and development of tomato genotypes investigated. Thus, the contribution of the genotype, isolate and genotype x isolate interaction for seeds germination was found to be 83.9; 10.0 and 4.2% for *F. oxysporum* CF, and 90.1; 6.8; 1.5% – for *A. alternata* CF, which indicates that for seeds germination, the genotypic factor had the greatest importance in the reaction to the mentioned pathogens.

In the variant with CF *F. oxysporum*, the contribution of their genotype, isolation and interaction in the source of variability of root and stem length was 1.8; 97.2; 0.7% and 4.0; 93.6; 2.0%, respectively, and for treatment with CF *A. alternata* – 3.1; 95.7; 0.8% and 7.5; 91.0; 0.9%, respectively.

Therefore, for the growth of the embryonic root and the stem in both variants, a major influence belongs to the isolate (Table 4).

Table 4. Bifactorial analysis of *tomato genotype x fungal pathogen* relationships

Source of variation	Freedom degree	Length of the root		Length of the stem		Germination	
		The average sum of the squares	Contribution in the source of variation, %	The average sum of the squares	Contribution in the source of variation, %	The average sum of the squares	Contribution in the source of variation, %
F. oxysporum							
Genotype	6	80.33*	1.8	65.17*	4.0	2015.2*	83.9
Isolate	3	4428.55*	97.2	1534.67*	93.6	241.5*	10.0
Genotype x isolate	18	30.35*	0.7	32.24*	2.0	101.0*	4.2
Random effects	56	14.80	0.3	7.27	0.4	45.8	1.9
A. alternata							
Genotype	6	137.85*	3.1	86.03*	7.5	2585.3*	90.1
Isolate	3	4318.84*	95.7	1044.31*	91.0	194.4*	6.8
Genotype x isolate	18	33.87	0.8	10.33	0.9	44.3	1.5
Random effects	56	20.01	0.4	7.12	0.6	45.9	1.6

*- $p<0.05$ (significance liver).

In connection with the above, the genotypic and phenotypic variations of the analyzed characters varied considerably depending on the species of the fungus, which was reflected in the fairly wide amplitude of the heritability coefficient – 0.60-0.95 (Table 5).

Table 5. Genetic variability and heredity of tomato growth organs in early ontogenesis at interaction with some fungal pathogens

Parameter	Length of the root		Length of the stem		Germination	
	F. oxysporum	*A. alternata*	*F. oxysporum*	*A. alternata*	*F. oxysporum*	*A. alternata*
σ^2_g	21.84	39.3	19.3	78.9	656.5	846.5
σ^2_{ph}	36.64	59.3	26.57	86	702.3	892.4
h^2	0.60	0.66	0.73	0.92	0.93	0.95
GCV, %	17.75	23.2	36.22	73.1	30.39	34.5
PCV, %	24.46	28.4	42.49	76.3	31.46	35.4
PCV – GCV, %	6.71	5.2	6.27	3.2	1.07	0.9
GA	16.7	18.6	12.77	13.5	27.57	29.9
GA, %	67.5	68.7	105.3	111.4	32.73	35.5

σ^2_g = genotypic variance; σ^2_{ph} = phenotypic variance; h^2 = coefficient of heritability in the broad sense; PCV = phenotypic coefficient of variation; GCV = coefficient of genotypic variation; GA = genetic advantage.

At the same time, it was observed that the h^2 values of seeds germination under the action of isolates of both fungi and stem length in interaction with *F. oxysporum* CF were much higher compared to other variants, which proves the genetic determinism of these characters.
The coefficient of genotypic variation was also medium or high - 17.75-73.3% for the studied characters, which proves the genetic nature of their variability. The difference between PCV and GCV was 0.9-6.71% and reflects the differentiated response of growth organs to the action of fungal isolates.

CONCLUSIONS

As a result of the analysis of the tomatoes perspective lines on *F. oxysporum* and *A. alternata* isolates extracted from sicked plants, it was found that germination, compared to 2 other characters - embryonic root length and stem length, was a factor with higher discriminant capacity.
Under the influence of fungal pathogens culture filtrates, in all of the cases there was inhibition of growth of the embryonic root and the stem, but the degree of reaction of the plants depended on the genotype, the analyzed character and the isolate of the fungus.
Bifactorial analysis of the variance found that for seeds germination, the genotypic factor was most the important in the reaction to *F. oxysporum* and *A. alternata*, and for the growth of embryonic root and stem in both variants a major influence belonged to the fungus isolate.
The high coefficient of heritability in the broad sense (h^2 = 0.60-0.95) indicates a good heredity of the studied characters in the interaction with the isolates of *F. oxysporum* and *A. alternata* fungi. We mention that the coefficient of genotypic variation was also medium or high - 17.8-73.1% for the studied characters, which proves the genetic nature of their variability.

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