THE REACTION OF SOME VALUABLE TOMATO SORTS (VARIETIES) TO FILTRATES OF *Fusarium* spp. AND *Alternaria alternata* CULTURES

MIHNEA Nadejda, LUPAŞCU Galina, GRIGORCEA Sofia, ZAMORZAEVA Irina

Abstract. There are presented the data regarding the reaction of some valuable tomato genotypes to culture filtrates of pathogenic fungi, which frequently have been isolated from plants with symptoms of disease. The significant influence of *Fusarium* spp. and *A. alternata* pathogens on the early stages of ontogeny of the tomato genotypes by the suppression of seed germination and growth of radicles and stemlets (sometimes by their stimulation) has been found. The varieties Tomis and Mihaela are less sensitive to the action of fungi and can be successfully used as donors of resistance to fusariosis and alternariosis. The significant ponderosity of the role of fungus species (*Fusarium* spp., *A. alternata*) and of 'tomato genotype x species of fungus' interaction, as the sources of variation of plant organs of growth, reveals the need for constant monitoring of the composition and virulence of fungi species causing root rot on tomato.

Keywords: tomato, varieties, culture filtrate, *Fusarium* spp., *Alternaria alternate.*

Rezumat. Reacţia unor soiuri valoroase de tomate la filtratele de cultură *Fusarium* spp. şi *Alternaria alternata*. Sunt prezentate date despre reacţia unor genotipuri valoroase de tomate la filtratele de cultură a patogenilor fungici *Fusarium* spp şi *Alternaria alternata*, frecvent izolaţi din plante cu simptome de boală. S-a constatat că patogenii *Fusarium* spp. şi *A. alternata* influenţează semnificativ ontogeneza timpurie a genotiputilor de tomate prin reprimarea germinaţiei seminţelor şi creşterii rădăciniţei şi tulpiniţei (uneori - prin stimularea acestora). Soiurile Tomiş şi Mihaela sunt mai puţin sensibile la acţiunea fungilor şi pot fi cu succes utilizate ca donatori de rezistenţă la fuzarioză şi alternarioză. Ponderea semnificativă a rolului speciei fungului (*Fusarium* spp., *A. alternata*) şi interacţiunii genotip de tomate x specie de fung în sursa de variaţie a organelor de creştere a plantelor, relevă necesitatea monitorizării constante a componenţei şi virulenţei speciilor de fungi care cauzează putregaiul de rădăcină la tomate.

Cuvinte cheie: tomate, soiuri, filtrate de cultură, *Fusarium* spp., *Alternaria alternata.*

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) occupy an important place in the global (BARONE et al., 2008) and national (BOTNARI & CEBOTARI, 2003) economy due to the high nutritional value of fruits. Different ways and modes of their consumption are possible: as fresh fruits or mixed with other vegetables, as sauces, stews, stuffed tomatoes, and as many other kinds of processed products.

Among biotic unfavourable factors affecting growth and development of tomato plants in the Republic of Moldova fungal pathogens *Fusarium* spp. that cause the rot of root and basal stem, plants wilt (LUPAȘCU, 2004; ROTARU, 2011), as well as *Alternaria* spp. that cause brown / black and dried spots on leaves, followed by early yellowing of leaves and premature drying (GRIGORCEA, 2014), must be considered.

In order to create valuable varieties, sustainable in terms of productivity and quality, genotypes need to include, as mandatory support, the resistance to biotic and abiotic unfavourable factors. That is why screening of perspective forms, concerning their reaction to these factors, is required. Moreover, the elucidation of complex resistance of already created varieties obligates to identify valuable genotypes with broad opportunities, which are involved in breeding programs as possible donors of resistance to known pathogens with high frequency and virulence (LUPAȘCU et al., 2008; MIHNEA et al., 2016).

Fungal diseases are usually controlled by chemical fungicides that cause undesired consequences for the human health and environment (GAVRILESCU & CHISTI, 2005). For these reasons, long-term use of resistant varieties is economically and environmentally advantageous (GUIMARAES et al., 2007; TSUTOMU et al., 2007).

The aim of this research was to identify tomato varieties with complex resistance to the *Fusarium* spp. and *Alternaria alternata* pathogens based on the reaction to the treatment with the pathogens culture filtrates.

MATERIAL AND METHOD

Six tomato varieties with high indexes of productivity and quality, created in the Institute of Genetics, Physiology and Plant Protection (IGPPP), were used as a material for research.

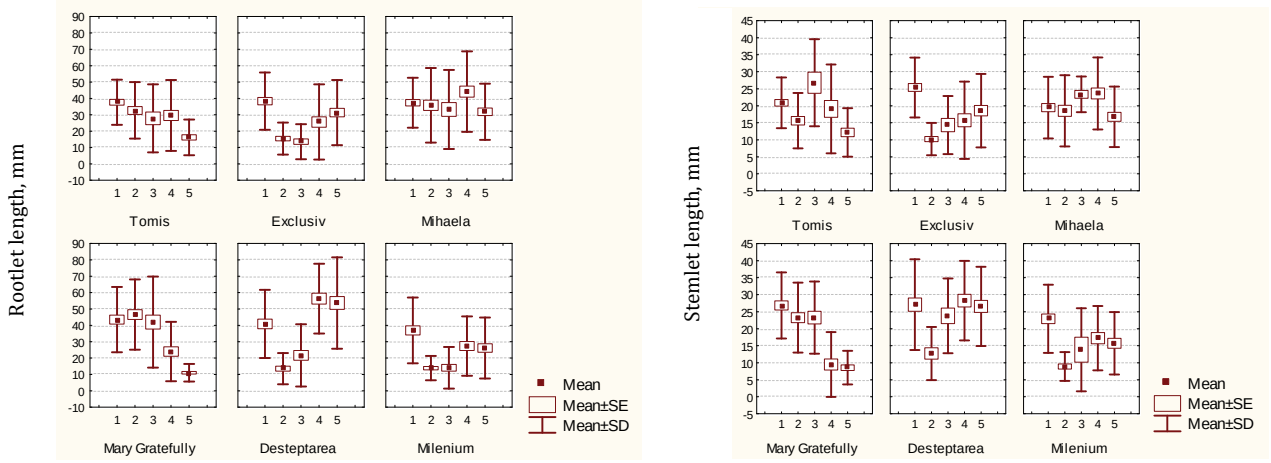
Culture filtrates (CF) of the fungi *F. oxysporum*, *F. solani*, *F. redolens*, and *Alternaria alternata* (isolated from infected tomato plants) were applied. They were prepared by the inoculation of mycelium into the Czapek-Dox liquid medium and subsequent cultivation at the temperature 22-24ºC for 21 days.

Tomato seeds were treated with CF of the fungi for 18 hours. Seeds, which were kept in distilled water, were used as a control. The cultivation of seedlings took place in Petri dishes on the filter paper, moistened by distilled water, at room temperature 22-24ºC for 6 days. The important characteristics like growth and development of tomato at the early stages of ontogeny (germination, rootlet and stemlet length) were taken as index-test of the reaction of plants.

Bifactorial analysis of the data ANOVA (package STATISTICA 7) was applied to assess the role of genotype, fungus species and their interaction as a source of variation in quantitative traits.

RESULTS AND DISCUSSIONS

The response of the varieties to the treatment of seeds with CF *F. oxysporum, F. solani, F. redolens, A. alternata* demonstrated strong differences in dependence on the analysed trait: germination, rootlet and stemlet length. The response to the infection fits in categories: lack of reaction, inhibition, stimulation (Fig. 1).



A B

Figure 1. The influence of the filtrates of *Fusarium* spp. and *Alternaria alternata* cultures to the radicles and stemlets growth of tomato seedlings.
Horizontally: 1 – control (H_2O), 2 – *F. oxysporum*, 3 – *F. solani*, 4 – *F. redolens*, 5 – *A. alternata*.

Germination. In the control, seed germination ranged within 85.0 ... 100.0%, so it was quite high. These values show the high quality of seeds and allow the opportunity to test reaction of plants. In most cases CF influence decreased germination. The most pronounced decrease was manifested in Tomis and Mihaela varieties in case of *F. solani* fungus where germination declined to 48.3 % and 55.0 %, respectively. The cases of the germination raise to 13.3 % ... 15.0 % were registered in Milenium and Desteptarea varieties with the influence of *A. alternata* CF.

Rootlet. In the control, the rootlet length was registered in the ranges of 36.9-43.5 mm in the studied varieties (Table 1). In variants with CF it has been shown repression in Tomis, Exclusive, Desteptarea, Milenium varieties (*F. oxysporum*); Tomis, Exclusive, Mihaela, Desteptarea, Milenium (*F. solani*); Tomis, Exclusive, Mary Gratefully, Milenium (*F. redolens*); Tomis, Exclusive, Mihaela, Mary Gratefully, Milenium (*A. alternata*), and significant stimulation in Mihaela (*F. redolens*), Mary Gratefully (*F. oxysporum*), Desteptarea (*F. redolens, A. alternata*).

Table 1. The influence of *Fusarium* spp. and *Alternaria alternata* culture filtrates on some characteristics of tomato growth and development.

No.	Variant	Germination, %	Rootlet length, mm		Stemlet length, mm	
			$x \pm m_x$	S	$x \pm m_x$	S
Tomis						
1	H_2O (control)	100	37. 7±1. 8	189. 1	20. 8±1. 0	55. 7
2	FC *F. oxysporum*	88. 3	32. 6±2. 4	297. 2	15. 6±1. 2*	66. 2
3	FC *F. solani*	48. 3	27. 9±3. 9*	431. 9	26. 8±3. 1	164. 1
4	FC *F. redolens*	81. 7	29. 6±3. 1*	468. 7	19. 1±2. 5	170. 1
5	FC *A. alternata*	78. 3	16. 2±1. 6*	119. 2	12. 2±1. 2*	50. 6
Exclusiv						
1	H_2O (control)	98. 3	38. 3±2. 3	305. 8	25. 4±1. 1	77. 4
2	FC *F. oxysporum*	91. 5	15. 5±1. 3*	95. 2	10. 2±0. 8*	22. 7
3	FC *F. solani*	71. 2	13. 6±1. 6*	113. 9	14. 3±2. 0*	72. 9
4	FC *F. redolens*	89. 8	25. 6±3. 1*	527. 1	15. 8±1. 9*	128. 7
5	FC *A. alternata*	100	31. 3±2. 6	395. 2	18. 6±1. 5*	116. 4
Mihaela						
1	H_2O (control)	100	37. 4±2. 0	230. 9	19. 5±1. 2	81. 8
2	FC *F. oxysporum*	90. 0	35. 8±3. 1	517. 6	18. 5±1. 6	109. 5
3	FC *F. solani*	55. 0	33. 3±4. 2	583. 3	23. 3±1. 2*	27. 5
4	FC *F. redolens*	88. 3	44. 2±3. 4	604. 2	23. 6±1. 6*	112. 0
5	FC *A. alternata*	90. 0	31. 8±2. 3	292. 3	16. 7±1. 3	78. 7
Mary Gratefully						
1	H_2O (control)	91. 7	43. 5±2. 7	399. 2	26. 8±1. 3	94. 6
2	FC *F. oxysporum*	92. 7	46. 6±92. 7	460. 7	23. 3±1. 4	105. 6
3	FC *F. solani*	78. 2	42. 0±4. 2	770. 9	23. 3±1. 8	112. 1
4	FC *F. redolens*	70. 9	24. 0±2. 9*	328. 0	9. 5±1. 6*	90. 9
5	FC *A. alternata*	81. 8	11. 0±0. 8*	28. 8	8. 6±0. 8*	24. 2

Desteptarea						
1	H_2O (control)	85	40. 9±2. 9	433. 8	27. 1±1. 9	178. 2
2	FC *F. oxysporum*	76. 5	13. 6±1. 5*	90. 4	12. 7±1. 7*	61. 0
3	FC *F. solani*	80. 4	21. 6±3. 0*	360. 8	23. 8±2. 3	120. 4
4	FC *F. redolens*	80. 4	56. 3±3. 3*	453. 2	28. 2±1. 8	136. 5
5	FC *A. alternata*	100	53. 6±3. 9*	776. 9	26. 6±1. 7	136. 8
Milenium						
1	H_2O (control)	86. 7	36. 9±2. 8	403. 3	22. 9±1. 4	100. 7
2	FC *F. oxysporum*	98. 1	13. 9±1. 0*	55. 1	8. 9±0. 8*	18. 0
3	FC *F. solani*	73. 1	14. 0±2. 1*	161. 2	13. 8±3. 7*	149. 0
4	FC *F. redolens*	84. 6	27. 3±2. 7*	329. 1	17. 2±1. 6*	89. 4
5	FC *A. alternata*	100. 0	26. 1±2. 5*	346. 0	15. 7±1. 5*	84. 3

Legend: * – distinction from the control at p≤0.05.

It should be mentioned that the reaction of different varieties to the same isolate was quite different. For example, the effect of *F. oxysporum* CF and *F. solani* CF on Exclusive, Desteptarea, Milenium varieties was strong, inhibition, on Mihaela variety - insignificant deviation from the control. Mary Gratefully variety demonstrated some stimulative effect of *F. oxysporum* CF on radicle growth, as well as *F. solani* CF that caused minor deviations. The culture filtrate of *F. redolens* influenced as stimulator Mihaela and Desteptarea varieties and as strong inhibitor Mary Gratefully variety. The analysis of 24 variants of CF treatment showed that the rootlet growth was inhibited in 20 cases and stimulated in 4 cases. Plants responded to the CF action not only by the decrease or increase of the growth but also by changing their heterogeneity. The presented data demonstrate that the value of variance (S) increased in 14 cases and decreased in 10 cases.

Stemlet. In the control, the variation of stemlet length was found within 19.5 ... 27.1 mm. In CF variants, different genotypes demonstrated sufficiently differential sensitivity. The influence of CF inhibited stemlet growth in 20 cases, and stimulated it significantly in 4 cases. The values of variation demonstrate that the CF influence increased plant heterogeneity in 10 cases, however it was less pronounced than in the case of radicle. Strong inhibition was shown under the influence of CF *F. oxysporum* at Exclusive, Desteptarea, Milenium varieties, *F. solani* CF inhibited Exclusive and Milenium, stimulated – Tomis and Mihaela. The culture filtrates of *F. redolens* influenced stimulatively the growth of Mihaela and Desteptarea stemlet, and inhibitively – Exclusive, Mary Gratefully, Milenium. Tomis showed lack of reaction. It should be mentioned that CF of *A. alternata* inhibited the growth of stemlet in all analysed varieties.

The bifactorial analysis of variance (Table 2) allowed finding that a species of fungus was the main source of rootlet and stemlet length variation, its contribution was 41.07 % and 58.58 %, respectively.

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

Source of variation	Degree of freedom	Mean sum of squares	Contribution in the source of variation,%
Length of radicle			
Tomato	5	8,100*	32. 49
Species of fungus	4	10,241*	41. 07
Tomato genotype x species of fungus	20	6,242*	25. 03
Random effects	1,424	351	1. 41
Length of stemlet			
Tomato genotype	5	1,210.2*	22. 00
Species of fungus	4	3,222.3*	58. 58
Tomato genotype x species of fungus	20	975. 1*	17. 73
Random effects	1,103	93. 5	1. 70

Legend: *-p≤0.05.

It should be mentioned that the genotype played an important role, too; its factorial weighting consisted of 32.49 % for the rootlet length and 22.00 % for the stemlet length. The interaction *genotype x species of fungus* was 25.03 % and 17.73 %, respectively, for both traits. The significant ponderosity of the role of fungus species (*Fusarium* spp., *A. alternata*) and interaction *genotype of tomato x species of fungus*, as sources of variation of plant organs of growth, reveals the need for constant monitoring of the composition of species of pathogens and their virulence.

CONCLUSIONS

1. It has been found that the *Fusarium* spp. and *A. alternata* pathogens influence significantly the early ontogeny of tomato genotypes by the repression of seed germination, growth of rootlets and stemlets (sometimes by their stimulation).

2. Mihaela and Tomis varieties are less sensitive to applied CF and can be successfully used as potential donors of resistance to fusariosis and alternariosis.

3. The significant ponderosity of the role of fungus species (*Fusarium* spp., *A. alternata*) and of *genotype of tomato x species of fungus* interaction, as the sources of variation of plant organs of growth, reveals the need for constant monitoring of the composition and virulence of fungi species causing root rot on tomato.

REFERENCES

BARONE A., CHIOSANO M., ERCOLANO M., GIULIANO G., GRANDILLO S., FRUSCIANTE L. 2008. Structural and Functional Genomics of Tomato. In: *International Journal of Plant Genomics*. DOI: 10.1155/2008/820274. (Accessed: May 30, 2015).

BOTNARI V. & CEBOTARI V. 2003. Legumicultura: Starea actuală şi perspectiva dezvoltării. *Analiza Sectorului Agricol*. Edit. CAMIB. Chişinău. 48 pp.

GAVRILESCU M. & CHISTI Y. 2005. Biotechnology - a sustainable alternative for chemical industry. In: *Biotechnol Advances*. **23**: 471-99. http://www.massey.ac.nz/~ychisti/Gavrilesc.pdf (Accesed March 02, 2016).

GRIGORCEA SOFIA. 2014. Influenta factorului matern asupra rezistentei la patogenii fungici şi elementelor de productivitate la tomate (*Solanum lycopersicum* L.). Autoref. tezei de dr. st. biologice. Institutul de Genetică, Fiziologie şi Protecţie a Plantelor. Academia de Ştiinţe a Moldovei. Chişinău. 37 pp.

GRIGORCEA SOFIA, LUPAŞCU GALINA, MIHNEA NADEJDA. 2011. Manifestarea alternariozei la soiuri şi hibrizi F_1 de tomate. In: *Structura şi funcţionalitatea sistemelor biologice - diversitate şi universalitate*. Edit. Print - Caro. Chişinău: 197-200.

GUIMARÃES ELCIO P., RUANE J., SCHERF B., SONNINO A., DARGIE J. 2007. Marker-Assisted Selection: current status and future perspectives in crops, livestock, forestry and fish. Rome FAO: 153-164.

LUPAŞCU GALINA. 2004. Protecţia plantelor de fuzarioze. In: *Protecţia plantelor. Realizări şi perspective. Conferinţa stiinţifico-practică „10 ani ai Centrului de Stat pentru Atestarea şi Omologarea Produselor de Uz Fitosanitar şi a Fertilizantilor”*. Edit. Tipografia Centrală. Chişinău: 31-32.

LUPAŞCU GALINA, ROTARU LUDMILA, MIHNEA NADEJDA**,** GAVZER SVETLANA. 2008. Oportunităţi de *screening* al rezistenţei genotipurilor de tomate la fuzarioza radiculară. In: *Probleme actuale ale geneticii, fiziologiei şi ameliorării plantelor*. Edit. Tipografia Centrală. Chişinău. 105-110.

MIHNEA NADEJDA, BOTNARI V., LUPAŞCU GALINA. 2016. Tomato Varieties with High Indices of Productivity and Resistance to Environmental Factors. In: *Ekin Journal of Crop Breeding and Genetics*. **2**(1): 15-22. http://ww2.ekinjournal.com/?folio=7POYGN0G2. (Accesed: 12 March, 2016).

ROTARU L. 2011. *Particularităţile controlului genetic al rezistenţei tomatelor la fuzarioza radiculară.* Autoref. tezei de dr. ştiinţe biologice. Edit.Primex-COM. Chişinău. 20 pp.

TSUTOMU A., TAKAHASHI H., KODAMA M., TEREOKA T. 2007. Tomato as a model plant for *plant-pathogen* interactions. In: *Plant Biotechnology*. Edit. J - STAGE. **24**: 135–147. https://www.jstage.jst.go.jp/article/plantbiotechnology/24/1/24_1_135/_pdf (Accesed: 12 March, 2016).

Mihnea Nadejda, Lupaşcu Galina, Grigorcea Sofia, Zamorzaeva Irina
Institute of Genetics, Physiology and Plant Protection of the Academy of Sciences of Moldova.
E-mail: mihneanadea@yahoo.com
E-mail: galinalupascu@gmail.com
E-mail: sofinel@mail.ru

Received: March 29, 2016
Accepted: July 20, 2016