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Original scientific paper

REACTION OF DIFFERENT TOMATO CULTIVARS TOWARD RACE 1 OF *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

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The aim of this study was to examine the reaction of different tomato cultivars towards race 1 of *Fusarium oxysporum* f. sp. *lycopersici*. The researched tomato cultivars were: Adonis, Gružanski zlatni, Jasmin crveni, Narvik SPF and SP109, breeding lines Hom-3, Hom-4, L-4, S-49, S-31, S-35, SPA, SPR,V-100, 34/56, 93/10 and 93/16. Reaction to pathogen were tested in F_1 generation, as well, in combination of 93/16 x V-100, S-35 x L-4, S-49 x SPA, 34/56 x SPR, S-49 x Hom-4, 93/16 x Hom-3 and 93/10 x S-31. Plants were inoculated by submerging wounded roots in to pathogen suspension. Disease was assessed 30 days after inoculation. After evaluation 15 cultivars expressed resistance toward this pathogen. Among

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them 93/10 x S-31, Hom-3 and 93/10 had average disease rating 1,0. Other genotypes had average disease ratings as follows: Narvik SPF and S-49 (1,1), 34/56, Hom-4 and S-49 x Hom-4 (1,2), while Adonis, 93/16 x Hom-3, 34/56 x SPR and 93/16 x V-100 had average disease rating 1,3. Cultivars SPR and 93/16 had 1,7 ADR, meanwhile Sp-109 had 1,8 average rating. Genotypes SPA (2,1), S-49 x SPA (2,1), V-100 (2,2) and L-4 (2,6) belong to the group of tolerant genotypes who did not expressed typical symptoms of chlorosis and wilt of plant but expressed some level of necrosis of xylem in lower part of plants. Most sensitive to fusarium wilt were S-35 x L-4 (3,3), S-35 (3,5), S-31 (3,6), Gružanski zlatni (3,8) and Jasmin Crveni with average disease rating of 4,0.

Key words: control, cultivars, *Fusarium oxysporum* f. sp. *lycopersici*, race 1, resistant, tomato

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill.) is one of the most important vegetable crops worldwide that could be used in many different ways, fresh as well as processed (GLOGOVAC *et al*., 2010; RADZEVIČIUS *et al*., 2009). Due to expansion of tomato production large number of different tomato varieties is being grown in Serbia, which caused apperence of different disease pathogens that threaten this production (MILIJAŠEVIĆ *et al*., 2009).

In tomato production, high yield and fruit quality are very important (ĐORĐEVIĆ *et al*., 2010; ZDRAVKOVIĆ *et al*., 2010, 2011a). Yield can be jeopardized by numerous pathogens. Therefore breeding for resistance to pathogens is of essential importance (ZDRAVKOVIĆ *et al*., 2011b). Soil fungi *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen (FOL), causer of fusarium wilt of tomato, is one of the most devastating and therefore economically important pathogen of tomato (BALAŽ *et al.,* 2009). This pathogen may cause great losses, especially on sensitive varieties and under favourable weather conditions. Once contaminated, soil remains contaminated indefinitely (AGRIOS, 2005). There are three identified races of this pathogen in the worlds up till now. The most common are races 1 and 2, while race 3 has limited geographical distribution, at the moment (GALE *et al*., 2003; REIS *et al*., 2005). At the same time the pathogen has been identified, the genes bearers of resistance in some tomato genotypes have been detected. Tomato cultivars resistant to race 1 contain gene *I*, while those resistant to race 2 contain gene *I-2*. Cultivars that contains gene *I-3* are resistant to race 3 of this pathogen (SCOTT *et al*., 2004; HEMMING *et al*., 2004).

In order to control FOL the most used substance was the methyl-bromide, but it has been banned by Montreal protocol due to its harmful effect to the environment. The study of other chemical substances did not give satisfactory level of efficiency for none of the researched active matters (IVANOVIĆ and IVANOVIĆ, 2007). Having in mind this fact on one hand and the need for environmental friendly and ecologically sustainable tomato production on the other it is necessary to find and develop as many strategies as possible in order to control fusarium wilt of tomato which will

meet these requirements. The implementation of the genes – carriers of resistance (*I* gen) is one of the strategies that could prevent or at least reduce the risks of yield losses in production of such important culture (DJORDJEVIC *et al.*, 2011). In order to create hybrids or varieties that are carriers of resistance gene it is necessary to test reaction of existing material to race 1 and establish the potential of existing genotypes that can be used in controlling this pathogen.

The aim of this research was to examine the reaction of different tomato cultivars, breeding lines and F_1 generation of combined breeding lines to race 1 of *Fusarium oxysporum* f. sp. *lycopersici* in order to review the current potential and set the direction of further selection.

MATERIALS AND METHODS

The pathogen was isolated from tomato plants with characteristic symptoms of fusarium wilt. After gaining a clean culture by method of single germinated conidia, pathogen was identified as *Fusarium oxysporum* with morphological methods (LEVIĆ, 2008). By applying the molecular methods and PCR it has been identified as race 1 of tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* by method LIEVENS *et al.* (2009). Pathogen is being kept in the collection of phytopathogenic fungi on PDA at 4°C in refrigerator for next use.

In order to test reaction toward race 1 of *F. oxysporum* f.sp. *lycopersici* 24 tomato genotypes were inoculated. Five of them were cultivars with known sensitivity/resistance reaction (Adonis, Narvik SPF, SP-109, Gružanski zlatni and Jasmin crveni), 12 were breeding lines (93/16, V-100, S-35, L-4, S-49, SPA, 34/56, SPR, Hom-4, Hom-3, 93/10 and S-31) and seven F_1 hybrids (93/16 x V-100, S-35 x L-4, S-49 x SPA, 34/56 x SPR, S-49 x Hom-4, 93/16 x Hom-3 and 93/10 x S-31).

For the purpose of inoculation pathogen has been grown on PDA and kept for 15 days at 24°C in thermostat. After this period the suspension has been made by rinsing of mycelia with distilled water through sterile gauze (5x5cm). The concentration of suspension of 10^8 conidia/ml has been determined by hematocytometer.

Seeds were sown in styrofoam trays with 103 cells, filled with sterile substrate. When the plant had four true leaves completely developed they have been removed from containers and the root was washed in order to be cleaned from substrate. The apical sector of root system, about 2 cm of it, was removed with scissors (GALE *et al.*, 2003; REIS and BOITEUX, 2007). After that, ten plants from each group have been submerged in pathogen suspension for 6 minutes. Ten plants submerged in distilled water for 6 minutes were used as control. After inoculation plants were planted in pots of 19cm diameter in sterile substrate and kept in glass house. Disease was assessed 30 days after inoculation using an modified ordinal scale (1 – 5) by REIS and BOITEUX (2007) where 1 = plant free of symptoms; 2 = plant without wilt symptoms but present conspicuous vascular browning; 3 = plant showing vascular browning with wilting symptoms or with chlorosis; 4 = severe wilting associated with the presence of foliar necrosis and chlorosis, and 5 = dead plant. Cultivars with average disease ratings in range of 1,0 – 2,0 were consider

resistant (R), from 2,1 – 3,0 were consider as tolerant (T) and cultivars with average disease ratings grater than 3,1 were considered susceptible (S).

Experiment has been set in totally random design with two replications. Data was proceeded in MATLAB Ver. 7.0 by applying variance analysis and differences were compared using Duncan Multiple Range test for the level of significance 0.05.

Cluster analysis was used as selection criteria to identify the divergence of tested genotypes related to phenotypic reaction of tomato plants to pathogen. For distance measuring Euclidean distance was used (STATISTICA 8.0; StatSoft, INC. (2007), data analysis software system, www.statsoft.com).

RESULTS AND DISCUSSION

Seven to ten days from inoculation the first symptoms were noticed on S-35 x L-4, Gružanski zlatni and C. Jasmin, as light chlorosis and wilt followed by intensive wilt and chlorosis. Most of other varieties and hybrids did not show visible symptoms of wilt and chlorosis at that time. Plants had normal colour and turgor or much less intensive symptoms. Only after observing the cross section of stem, necrotic changes of vascular tissue, characteristic for this pathogen could be found.

Result of this study shows that from all observed genotypes, 15 showed resistance to this pathogen. 93/10 x S-31, Hom-3 and 93/10 had average disease rating 1.0, and they were considered resistant to race 1. Genotypes, such as, Narvik, S-49, 34/56, Hom-4, S-49 x Hom-4, Adonis, 93/16 x V-100, 34/56 x SPR, 93/16 x Hom-3, SPR, 93/16 and SP-109 are also in the group of resistant cultivars to race 1 of *F. oxyporum* f. sp. *lycopersici*, based on the results. The most sensitive was Jasmin Crveni followed by Gružanski zlatni, S-31, S-35 and S-35 x L-4 (table 1). F_1 hybrid S-49 x SPA, SPA, V-100 and L-4 although tolerant (without characteristic symptoms of wilt and chlorosis), manifested xylem necrosis located on lower part of the tomato stem, near the ground. Vascular tissue did not expressed dark necrotic colour typical for total necrosis which was found at most sensitive genotypes. In this case the xylem tissue was more light brown colour that points that necrotic changes of vascular tissue has began. This necrosis did not lead to wilt and chlorosis and did not even effect the growth of plants. Since the necrosis of vascular tissue could affect quality and quantity of tomato fruit due to possible decrease of water flow, yield of these cultivars will be the subject of further research.
(Table 1.)

Hybrid combinations 93/16 x Hom-3, 34/56 x SPR and S-49 x Hom-4 expressed resistance toward race 1 of fusarium wilt of tomato as well as parental lines. Hybrid combination 93/10 x S-31 had expressed resistance reaction toward this pathogen even though parental line S-31 were sensitive to this pathogen. *I* gene that caries resistance toward race 1 of this pathogen, is dominant which implies monogenic character of resistance regulation. Combination S-35 x L-4 had sensitive reaction, although father line L-4 expressed tolerance to this pathogen when it was tested alone. Sensitive reaction of combination S-35 x L-4 although father line L-4 expressed tolerance tested alone. Reaction of hybride S-49 x SPA was tolerant although parental line S-49 was resistance toward this pathogen (Tab 2).

Table 1: Reaction of tomato genotypes inoculated by race 1 of Fusarium oxysporum f. sp. Lycopersici

Genotipe	Average Disease Rating (ADR)*	Diferences between means **	Reaction***
Reaction of breeding lines of tomato inoculated with race 1 of *Fusarium oxysporum* f. sp. *lycopersici*			
Adonis	1,3	j k	R
Gružanski zlatni	3,8	a b	S
Hom-3	1,0	k	R
Hom-4	1,2	k	R
Jasmin Crveni	4,0	a	S
L-4	2,6	f	T
Narvik SPF	1,1	k	R
S-31	3,6	a b c	S
S-35	3,5	b c d	S
S-49	1,1	k	R
SPA	2,1	g h	T
SPR	1,7	h i j	R
SP – 109	1,8	g h i	R
V-100	2,2	f g	T
34-56	1,2	k	R
93-10	1,0	k	R
93-16	1,7	h i j	R
Reaction of tomato hybrids inoculated with race 1 of *Fusarium oxysporum* f. sp. *lycopersici*			
93/16 xV-100	1,3	j k	R
S-35 x L-4	3,3	c d e	S
S-49 x SPA	2,1	g h i	T
34/56 x SPR	1,3	j k	R
S-49 x hom-4	1,2	k	R
93/10 x S-31	1,0	k	R
93/16 x hom-3	1,3	j k	R

* Average of 10 plants. Plants were evaluated using an ordinal scale ranging from 1-no symptoms to 5-dead plants

** Values with different letters are significantly different according to Duncans Multiple Range test for level of significance P=0,05

*** Varieties with disease ratings between 1,0 – 2,0 were consider resistant (R), with disease ratings between 2,1 – 3,0 were considered tolerant (T) and higher than 3,1 were considered susceptible (S)

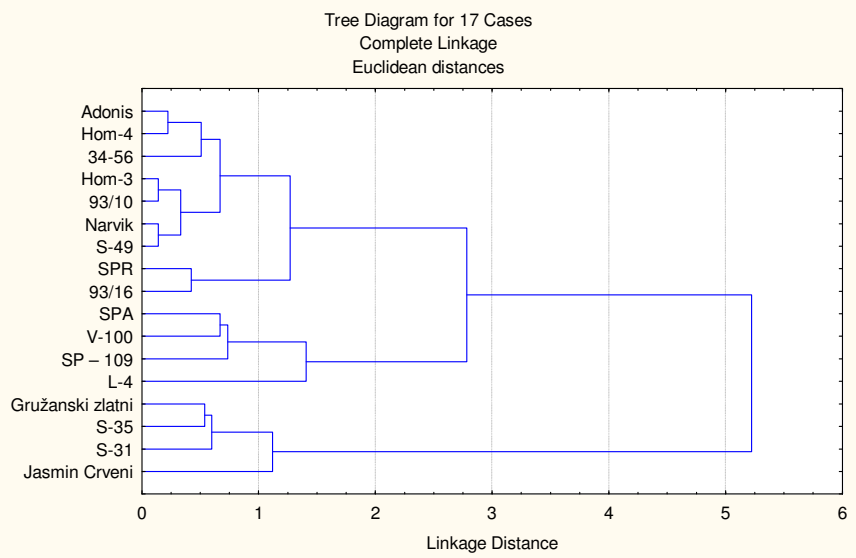
Table 2. Hybrids and parental lines of hybrids for testing

Hybrid	Reaction	Father ♂	Reaction	Mother ♀	Reaction
93/16 xV-100	R	V-100	T	93/16	R
S-35 x L-4	S	L-4	T	S-35	S
S-49 x SPA	T	SPA	R	S-49	R
34/56 x SPR	R	SPR	R	34-56	R
S-49 x hom-4	R	Hom-4	R	S-49	R
93/10 x S-31	R	S-31	S	93/10	R
93/16 x hom-3	R	Hom-3	R	93/16	R

According to Duncans Multiple Range test that compares mean values of ADR of individual genotypes, within the group of genotypes that were characterised as sensitive (S-35 x L-4, Gružanski zlatni, S-35, S-31 and Jasmin crveni), Jasmin crveni was not significantly different from Gružanski zlatni and S-31, but it was significantly different from S-35 and S-35 x L-4. Within the group of genotypes characterised as tolerant (SPA, V-100, SP-109 and L-4) genotype L-4 was significantly different from SPA and SP-109, meanwhile it was not significantly different from V-100 in reaction toward the pathogen. Also, we observed different statistical signifficance between the means among the resistant genotypes. Genotype 93/16 was significantly different from all of the resistant genotypes except SPR genotype. Meanwhile, means of all other genotypes that expressed resistance toward race 1 of this pathogen are not significantly different (Tab 1).

Cluster analysis indicates that there are two groups of genotypes based on their reaction toward race 1 of *Fusarium oxysporum* f. sp. *lycopersici*. Smaller group include four genotypes that expressed sensitive reaction toward this pathogen (Gružanski zlatni, S-35, S-31 i Jasmin crveni). All of the other genotypes belong to second group divided in two sub-clusters. In one sub-cluster there are genotypes that are marked as tolerant toward observed pathogen (SPA, V-100, SP-109 and L-4). Second sub-cluster includes genotypes that are resistant toward race 1 of fusarium wilt of tomato (93/16, SPR, S-49, Narvik, 93/10, hom3, 34-56, hom-4 i Adonis) (Graph 1).

Hybrid S-35 x L-4 is expressed sensitive reaction and it was created crossing of sensitive x tolerant parent lines. Hybride 93/16 x V-100 expressed resistant reaction toward this pathogen and it is a product of hybridization of resistant x tolerant meanwhile hybrid combination of S-49 x SPA expressed tolerant reaction evendough it was created crossing of resistant x tolerant genotypes. Remained combinations 34/56 x SPR, S-49 x Hom-4, 93/10 x S-31 and 93/16 x Hom-3 represent combinations of resistant x resistant genotypes (Tab. 1).



Graf 1. Dendogram of genetic distance of genotypes (Euclid distance) based on resistance toward race 1 of *Fusarium oxysporum* f. sp. *lycopersici*

Fusarium wilt of tomato, caused by *Fusarium oxysporum* f. sp. *lycopersici*, affects tomatoes worldwide. One of the aims of tomato breeding is development of cultivars resistant to *F. oxysporum* f. sp. *lycopersici.* This represent the effective strategy regarding controlling of fusarium wilt of tomato as well as preservation of environment.

In breeding programs worldwide of hybrids and varieties resistant and tolerant to race 1 and other two races of this pathogen is present for quite some time (TAKKEN and REP, 2010). Since the race 1 appeared first, i.e. it was the first to be proved and identified, resistance gene to this race, *I* gene was also first identified in *Solanum pimpinellifolium* 1940s (TAKKEN and REP, 2010). Plant material sensitive to race 1 is being researched for many years in order to find the suitable bearers of *I* gene and include them in selection. This is why *I* gene was most deployed gene in tomato cultivars (SCOTT and GARDNER, 2006).

In this research we studied reaction of 24 genotypes toward race 1 of *F. oxysporum* f.sp. *lycopersici*. Resistant reaction expressed 15 genotypes, tolerant were 4, and sensitive reaction expressed 5 genotypes. Hybrid 93/10 x S-31 expressed resistant reaction to race 1 of this pathogen although parental line S-31 expressed sensitive reaction to the same race. It is clear that the *I* gene that carrier of resistance toward race 1 of this pathogen is dominant and that the inheritance of resistance of tomato to this pathogen is monogenic (PANTHEE and CHEN, 2010). Hybrid combination S-49 x SPA expressed tolerant reaction toward pathogen even though parental line S-49 was resistant. Tolerant reaction indicates a level of resistance

toward the pathogen. Fungal races contain dominant virulence genes that correspond to dominant resistance genes in the cultivars that can not be infected (SCOTT and GARDNER, 2006). Breeding lines of Institute for vegetable crops posses resistance toward race 1 of *F. oxysporum* f. sp. *lycopersici* and they were crossed and tested in order to obtain new commercial hybrid combinations of tomato that will be resistant to this pathogen. AMINI (2009) tested 24 cultivars of tomato and classified them in groups of resistant-tolerant –sensitive toward race 1 of this pathogen. Also, he defined one more group of intermediately resistant. In that way he explained more closely levels of resistance. The results of our research are in accordance with the above and with studies of other researchers dealing with problems of tomato resistance to *Fusarium oxysporum* f. sp. *lycopersici* (SCOTT *et al*., 2004). Results of our study are important since in breeding programs designed to introduce new traits into breeding lines, such as pathogen resistance, bioassays are needed to assess the inheritance of the introduced trait in the breeding lines (EL MOHTAR *et al*., 2007).

Cluster analysis distributed genotypes in to three groups (resistant, tolerant and sensitive) based on the parameters that indicate sensitivity/resistance toward race 1 of *Fusarium oxysporum* f. sp. *lycopersici*. Heterogeneity of tested breeding lines within sub-clusters indicate differences between them that gives wide range of possibilities for choosing certain genotypes that can be included in breeding programs in order to obtain resistant hybrids of tomato. Taking in consider that the both ways of analysis, cluster and Duncans Multiple Range test have their advantages and disadvantages it can be recommended combined analysis for selection appropriate genotypes that will be included in breeding tomato resistant to race 1 of *Fusarium oxysporum* f. sp. *lycopersici* (SINGH *et al*., 1991).

Based on results of this study we found that higher number of observed cultivars are resistant or tolerant to race 1 pathogen *F. oxysporum* f. sp. *lycopersici*. This is an important fact if we have in mind the historic development and appearance of certain races of this pathogen in the world. After identifying *I* gene, carrier of resistance to race 1 of fusarium wilt and its implementation in selection of resistant cultivars, race 2 of this pathogen appeared and spread quickly at the begging of 1970's (JONES and CRILL, 1974). Similar happened after identification and appliance of gene *I-2* bearer of resistance to race 2, when race 3 appeared in 1979s (SCOTT *et al*., 2004). Therefore sensitivity should be tested for other races of this pathogen since resistance to race 1 usually does not mean resistance to other races which could be a reason of spreading of other races of this pathogen. Reaction of different cultivars to races 2 and 3 of tomato fusarium wilt will be the subject of further research.

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REAKCIJA RAZLIČITIH KULTIVARA PARADAJZA PREMA RASI 1 *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

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Cilj ovog rada je da se ispita reakcija različitih kultivara paradajza u odnosu na rasu 1 *Fusarium oxysporum* f. sp. *lycopersici*. Ispitivani genotipovi paradajza su sorte: Adonis, Gružanski zlatni, Jasmin crveni, Narvik SPF i Sp-109, selekcione linije Hom-3, Hom-4, L-4, S-49, S-31, S-35, SPA, SPR, V-100, 34/56, 93/10 i 93/16. Takođe, ispitana je i reakcija F_1 generacije sledećih kombinacija 93/16 x V-100, S-35 x L-4, S-49 x SPA, 34/56 x SPR, S-49 x Hom-4, 93/16 x Hom-3 i 93/10 x S-31. Biljke su inokulisane potapanjem povređenog korena u suspenziju patogena. Intenzitet oboljenja je ocenjivan nakon 30 dana posle inokulacije. Nakon evaluacije 15 genotipova je pokazalo rezistentnu reakciju prema ovom patogenu. Među njima 93/10 x S-31, Hom-3 i 93/10 imali su prosečnu vrednost oboljenja (ADR) 1,0. Ostali genotipovi su bili sledećih ADR vrednosti: Narvik SPF i S-49 (1,1), 34/56, Hom-4 i S-49 x Hom-4 (1,2), dok su Adonis, 93/16 x Hom-3, 34/56 x SPR i 93/16 x V-100 imali ADR vrednost 1,3. Genotipovi SPR i 93/16 imali su 1,7 ADR, dok je Sp-109 imao 1,8 prosečnu vrednost. Genotipovi SPA (2,1), S-49 x SPA (2,1), V-100 (2,2) i L-4 (2,6) svrstani su u grupu tolerantnih genotipova koji nisu imali tipične simptome hloroze i uvenuća ali se kod njih razvio određeni nivo nekroze ksilema lokalizovane na donji deo stabla. Kao najosetljiviji prema ovom patogenu pokazali su se S-35 x L-4 (3,3), S-35 (3,5), S-31 (3,6), Gružanski zlatni (3,8) i Jasmin Crveni sa prosečnom vrednošću oboljenja 4,0.

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