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EFFECT OF RACE 3 OF *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* ON SOME TOMATO CULTIVARS

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SUMMARY

Aim of this study is to determine the impact of race 3 of fusarium wilt on some tomato cultivars if it occurs in Serbia. For this purpose eleven tomato cultivars were inoculated with this pathogen: 129 – sprin, Balkan F_1, Danubius F_1, Jasmin crveni, M – 7, M - 10, Marko F_1, Nada F_1, Narvik, Šampion F_1, Zlatni Jubilej F_1 by applying classic method of inoculation by submersing the injured root in fungi suspension. Disease was assessed 30 days after inoculation using an ordinal scale range from 1 – 5. After assessment, Nada F_1 and 129-Sprin had lowest average disease rating (2,9), and marked as tolerant. All the other cultivars were consider susceptible with ADR values higher than 3,0 in the following order: Narvik (3,6), Šampion F_1 (3,8), M-7 (3,9), Z. Jubilej F_1 (4,1), C. Jasmin (4,2), Danubius F_1 (4,4), Balkan F_1 (4,5), Marko F_1 and M-10 (4,6). Based on these results we can conclude that if the race 3 of *Fusarium oxysporum f. sp. lycopersici* occur in Serbia it could seriously jeopardize tomato production.

Key words: race 3, *Fusarium oxysporum* f. sp. *lycopersici*, resistance, tomato, breeding

INTRODUCTION

Cultivated tomato (*Lycopersicon esculentum* Mill.) is one of the world's most important crops due to the high value of its fruits both for fresh market consumption and in numerous types of processed products (Giovanni et al., 2004). World volume of production has increased approximately 10% since 1985, reflecting a substantial increase in dietary use of the tomato. One of the main constraints to tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production (Tanyolaç and Akkale, 2010).

Fusarium wilt of tomato (*Lycopersicon esculentum* L.) caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) is one of the most impor-

tant and widespread diseases of tomato. It is a soil-borne fungus specilized for colonization of tomato. It produces chlamidospores that remain viable for long period of time, and because of that it was first described by Massee (1895) as the „sleepy disease" (*cit.* Huang-Cheng and Lindhout, 1997). This pathogen has three identified races, till now, and races 1 and 2 have a world wide distribution, whereas race 3 has a more limited geographical range (Reis et al., 2005). As the most effective mean for control of this pathogen methyl-bromide was used. But because of the harmful effects of this substance on the ozone layer it was banned for use (Gullino et al., 2002). Since than, methyl-bromide does not have adequate replacements (Bell, 2000; Ioannou, 2000; Ivanović and Ivanović, 2007).

Due to the inefficiency of fungicides and other conventional control methods, considerable breeding efforts have been directed toward the development of resistant tomato cultivars. Three major resistance loci have been genetically characterized in *Lycopersicon* species and all of them have been incorporated into comercial cultivars (Reis et al., 2004). Resistance genes conferring resistance to Fol race 1 (*I* gene) have been identified and mapped to chromosomes 11 (Bohn and Tucker, 1939; Paddock, 1950) and 7 (Sarfatti et al., 1991). The *I-2* gene, conferring resistance to Fol race 2, lies within a cluster of seven similar genes on the long arm of chromosome 11 (Laterrot, 1976; Segal et al., 1992). Gene *I-3* provides resistance against Fol races 1, 2 and 3 and was mapped to chromosome 7 (Bournival et al., 1989, 1990; Scott and Jones, 1989). Due to limitted geographical distribution of race 3 resistant genes are also limitted mostly to those regions.

Taking in consider facts that race 3 of Fol is very agressive and that it has not been proven to be present in Serbia, it would be of great importants to be prepare for its eventual occurence. The aim of this research is to investigate effect of race 3 of Fol on some of the tomato cultivars in order to simulate what would happen if this race 3 appear in Serbia and what would be the consequences.

MATERIAL AND METHODS

Isolate of pathogen was provided by Dr Bart Lievens, Sciencia Terrae Research Institute, Belgium. Pathogen is being kept in phytopathogen fungi collection on PDA at 4°C in refrigerator until further use.

Tomato cultivars from the Institute for Vegetable Crops, Smederevska Palanka: 129 – sprin, Balkan F_1, Danubius F_1, Jasmin crveni, M – 7, M - 10, Marko F_1, Nada F_1, Narvik, Šampion F_1, Zlatni Jubilej F_1 have been inoculated.

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 (Đorđević et al., 2012).

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. Control was ten plants submerged in distilled water also for 6 minutes. After that period 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

Table 1. Reaction of tested tomato cultivars and hybrids to race 3 of *Fusarium oxysporum f.sp. lycopersici*

Genotype	Category*	Duncan's Multiple range test **	Susceptibility***
129 – sprin	2,9	h	T
Balkan F_1	4,5	a	S
Crveni Jasmin	4,2	a	S
Danubius F_1	4,4	a	S
M – 7	3,9	d e	S
M – 10	4,6	a	S
Marko F_1	4,6	a	S
Nada F_1	2,9	h	T
Narvik	3,6	d e f g	S
Šampion F_1	3,8	d e f	S
Zlatni jubilej F_1	4,1	d	S

* 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=0,01
*** 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)

= 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 (ADR) 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 higher 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 Multi Range test for the level of significance 0,01.

RESULTS AND DISCUSSION

About 7-10 days after inoculation on all of tested cultivars and hybrids first symptoms occured, expressed as wilting in the wormest part of the day. As the time passed plants expressed more severe wilting with occurence of chlorosis, defoliation of lower leaves and even death. After the final evaluation Nada F_1 and 129 – sprin expressed highest level of tolerance, among tested cultivars, with ADR value 2,9. Based on this value they were marked as tolerant (T). All of the other cultivars and hybrids were highly susceptible with ADR higher than 3,0. Highest value of ADR (4,6) had Marko F_1 and M-10 (Table 1.).

Occurence of symptoms of wilt were not as severe as it would be expected. In fact symptoms were at first moderate intesity and as the time passed simtoms were more intensive and resulted with high procent of dead plants. Even Balkan F_1, Marko F_1 and M-10 that had highest values of ADR initially did not express intensive wilt symptoms but at the end of research majority of plants were dead. Nada F_1 and 129-sprin expressed moderate symptoms of wilt and chlorisis of leaves but cross section showed necrotic changes of xylem.

Most of the cultivars and hybrids in our research reacted as susceptible toward race 3,

except Nada F_1 and 129-sprin that expressed symptoms characteristic for fusarium wilt but marked as tolerant. Cultivar and hybrids that expressed susceptibility toward this race are most likely laking of the *I-3* gene. The *I-3* gene conferring resistance to race 3 was discovered in *L. pennellii* accessions PI414773 (McGrath et al., 1987) and LA716 (Scott and Jones, 1989). At first *I-3* gene from LA716 was found to confer resistance to race 1 and 2 (Bournival et al., 1990) but in recent findings of Scott et al. (2004) indicate that this gene does not confer resistance to race 1 and 2 but other genes *I-1* and *I-2* previously reported by Sarfatti et al. (1991). Nada F_1 and 129-sprin may have *Tfw* gene for tolerance to race 3 of fusarium wilt and confers limited resistance to all thre races (Bournival et al., 1989; 1990). This assumption will be tested using molecular methods in further reseach. The severity of infection of tomato plants by race 3 of FOL is in accordance with results of Scott et al. (2004) as well as with Reis et al. (2004) that inoculated 94 different tomato cultivars with race 3 and observed on 64 cultivars high level of susceptibility with the same pattern of symptom develpment.

Our results are expected due to the fact that resistant cultivars are mostly located in regions of the world with reported race 3 (Reis et al., 2005; Scott et al., 2004). Larger number of cultivars, especialy ones located among „wild“ population, should be tested in order to find *I-3* or *Tfw* genes. This will be a subject for further research.

CONCLUSION

Due to the high mobility of people and goods it is very easy to introduce this and other pathogens especially on seeds. Based on the results of our experiment race 3 of *Fusarium oxysporum* f. sp. *lycopersici* might became economycally important disease if introduced in our region since race 3-resistant cultivars are not yet available. Further reseach should be performed and large population of domestic cultivars of tomato should be examined in order to find gene or genes that confer resistance to this race, and introgress them into breeding programs of cultivated tomato. This will allow the anticipation of potential problem that will at some point in future occure.

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UTICAJ RASE 3 *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* NA POJEDINE KULTIVARE PARADAJZA

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REZIME

Cilj ovog istraživanja je da se utvrdi kakav bi bio uticaj rase 3 fuzarioznog uvenuća paradajza na pojedine kultivare ukoliko bi se ova rasa pojavila u Srbiji. U te svrhe inokulisano je jedanaest kultivara ovim patogenom i to: 129 – sprin, Balkan F_1, Danubius F_1, Jasmin crveni, M – 7, M - 10, Marko F_1, Nada F_1, Narvik, Šampion F_1, Zlatni Jubilej F_1, primenom klasične metode inokulacije umakanjem povređenog korena u suspenziju gljiva. Nakon 30 dana rađena je procena pojave oboljenja upotrebom skale od 1 – 5. Nakon evaluacije, Nada F_1 i 129-Sprin su imali najnižu vrednost ADR-a (prosečni nivo oboljenja) (2,9), i obeleženi su kao tolerantni. Svi ostali kultivari smatrani su osetljivim sa vrednostima ADR višim od 3,0, po sledećem rasporedu: Narvik (3,6), Šampion F_1 (3,8), M-7 (3,9), Z. Jubilej F_1 (4,1), C. Jasmin (4,2), Danubius F_1 (4,4), Balkan F_1 (4,5), Marko F_1 and M-10 (4,6). Na osnovu ovih rezultata možemo zaključiti da ako bi se rasa 3 *Fusarium oxysporum* f. sp. *lycopersici* pojavila u Srbiji mogla bi značajno da ugrozi proizvodnju paradajza.

Ključne reči*:* rasa 3, *Fusarium oxysporum* f. sp. *lycopersici*, otpornost, paradajz, selekcija

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