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Original scientific paper

INFLUENCE OF TOMATO GENOTYPE TO PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT ACTIVITY AS REACTION TO EARLY BLIGHT

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Early blight is one of the most common and destructive tomato disease and it is caused by the fungus *Alternaria solani.* The aim of this paper was to screen the reaction of ten tomato genotypes (collection of the Institute of Field and Vegetable Crops) against natural infection of early blight. Tested genotypes showed significant differences in the disease occurrence on leaves but not on fruits. However, at the biochemical level, total phenolics (TP), tannins (TT), flavonoids (TF) and antioxidant activity in tomato fruits was significantly affected by genotype, disease occurrence and interaction of these two factors. According to obtained results, content of these secondary metabolites could be used as a one of the parameters in the evaluation of tomato resistance to EB.

Key words: tomato genotypes, early blight, polyphenol compounds, antioxidant activity

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a horticultural crop of great interest, has been widely consumed either fresh or processed (HELYES *et al.*, 2009; RAY *et al.*, 2011). Tomatoes are rich in food components with antioxidant activity and considered to be a source of carotenoids, in particular lycopene, ascorbic acid and phenolic compounds (GEORGE *et al.*, 2004; GLOGOVAC *et al.*, 2010; ILAHYA *et al.*, 2011; PINELA *et al.*, 2012). These compounds may play an important role and have protective effect to the human health (BORGUINI *et al.*, 2009) by inhibiting reactive oxygen species responsible for many important diseases (CROZIER *et al.*, 2009). However, secondary metabolites such as phenolic compounds are also very important in plants ecology and their adaptation to different biotic and abiotic factors. Phenolic compounds are part of plant defense system either as constitutive compounds or synthesized postinfectional. Those compounds accumulate in response to pathogen attack (EWANÉ *et al.*, 2012; MALENČIĆ *et al.*, 2012) and have significant scavenging activity of reactive oxygen species (WITZELL and MARTÍN, 2008).

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One of the most common and destructive tomato disease is early blight (EB) caused by the fungus *Alternaria solani*. Uncontrolled, the disease may cause severe defoliation, resulting in reduced fruit number and size. Control measures include use of resistant or tolerant cultivars, crop rotation, and eradication of weeds, keeping the plants growing properly and fungicides application (JONES *et al.*, 2006). Use of resistant cultivars is potentially the most economical control measure (CHAERANI and VOORRIPS, 2006). Identification of additional sources of resistance could facilitate the development of resistant cultivars (CHAERANI *et al.*, 2007).

The induction of phenolic compounds synthesis during pathogen attack was demonstrated in tomato leaf tissue (PEARCE *et al.*, 1998). The aim of this paper was to screen the reaction of ten tomato genotypes from the collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia to the natural EB infection. The reaction to the pathogen was evaluated through the level of leaf and fruit infection and synthesis of phenolic compounds and antioxidant activity in the infected and healthy fruits.

MATERIALS AND METHODS

Plant material

Ten tomato genotypes (Table 1) from the collection of the Institute of Field and Vegetable Crops were included in the trials. The tomato genotypes originate from eight countries.

Table 1. List of tested tomato genotypes

Collection number	Genotype	Origin	Plant growth type*	Time of maturity (days)
S1	Beefsteak	USA	2	125
S21	Robura	Slovakia	1	115
S25	Royal Ball	Italy	1	130
S35	Volovsko srce**	Serbia	2	135
S41	China 5	China	1	135
S43	Dansk export	Denmark	1	115
S84	Aniko	Ukraine	1	135
S101	Ficarazzi	Italy	2	130
Line	V9***	Serbia	2	120
Variety	Bačka	Serbia	1	135

*Plant growth type according to UPOV descriptors: 1-determinate, 2- indeterminate

**Local population

***V9 is registered in 2014 as new variety-Dunavski rubin in Republic of Serbia

A trial was conducted at experimental field of Vegetable Crops Department in Rimski Šančevi in 2011. The trial was designed according to randomized block system in 3 replicates. Four plants were evaluated in each replicate. Sowing for seedlings production in glass house was done at the end of March and the plants were transplanted in the first decade of May into the open field.

The first evaluation of the early blight was done by estimation of total leaf area infection per plant. The plants were in the fruiting stage (July, 20th). Evaluation of the disease intensity on fruits was done on 8-9th of August in their botanical maturity by the same scale. Disease severity was rated from 1 to 9 scale: 1 = asymptomatic; 2 = few small lesions; 3 = several small lesions; 4 = <10% of area with infection; 5 = 10-20%; 6 = 21-40%; 7 = 51- 80%; 8 = 81-99% of area with infection; 9 = plant dead/completely diseased fruit (POYSA and TU, 1996).

After disease assessment, fruits were divided into two groups: healthy and infected ones. In each group content of polyphenols, tannins and flavonoids as well as antioxidative activity was measured in four replications per genotype.

Extraction and determination of total polyphenols and tannins

Plant material (200 mg) was extracted with 70% aqueous acetone solution (50 mL) by sonication for 20 minutes in an ultrasonic bath at ambient temperature. The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed.

Total polyphenols were determined colorimetrically (Jenway 6505, UK) in the acetone extracts at 720 nm using Folin-Ciocalteu reagent (KROYER, 2004). Gallic acid (GAE) was used as a standard (covering the concentration range between 0.1 and 1.0 mg/mL). The results are expressed as mg of GAE/g fruit weight (FW).

Total tannins content was determined by the Folin-Ciocalteu procedure, after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrrolidone) (HAGERMANN *et al.*, 2000). Calculated values were subtracted from total polyphenol contents, and total tannin contents were expressed as milligrams of GAE per gram of FW.

Extraction and determination of flavonoids

Total amount of flavonoids was determined after extraction of 1 g of dry plant material with 20 mL of extracting solvent methanol water-acetic acid (140:50:10 by volume), for 60 minutes (QUETTIER *et al.*, 2000). Total flavonoids content was calculated as a rutin equivalent from the calibration curve of rutin standard solutions and expressed as milligrams of rutin per gram of FW.

Measurement of antioxidant activity

The potential antioxidant activity of the test samples have been assessed on the basis of scavenging activity of the 10% aqueous acetone tomato fruit extracts of the stable DPPH free radicals (KUMARASAMY *et al.*, 2007). DPPH-radical scavenging activity was expressed as IC50 concentration where 50% inhibition of the DPPH radical is obtained.

Statistical analysis

Obtained data were analyzed using software STATISTICA ver. 12 (StatSoft, Inc., USA). Values for leaf and fruit tomato infection were analyzed by non parametric statistics Kruskal-Wallis test. Results for biochemical parameters were tested by analysis of variance followed by comparison of means by Fisher LSD test ($P<0.05$). Correlation coefficients were calculated according to Pearson.

RESULTS AND DISSCUSION

Leaf and fruit infection

Obtained results showed some differences in the EB intensity on leaves between tested genotypes. The medians of intensity of leaf infection were ranged from 1 to 2, which indicated only few small lesions on the leaves (Figure 1). The highest disease infection on leaves was 5 (20% of leaf area infection). Significant differences were found between genotypes Royall Ball (asymptomatic leaves) and Beefsteak.

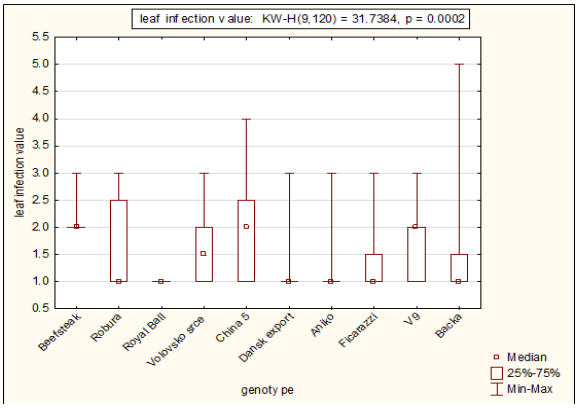

Figure 1. Intensity of early blight on the tomato leaves

Infection on the fruits was very low and there were no significant differences between genotypes (Figure 2).

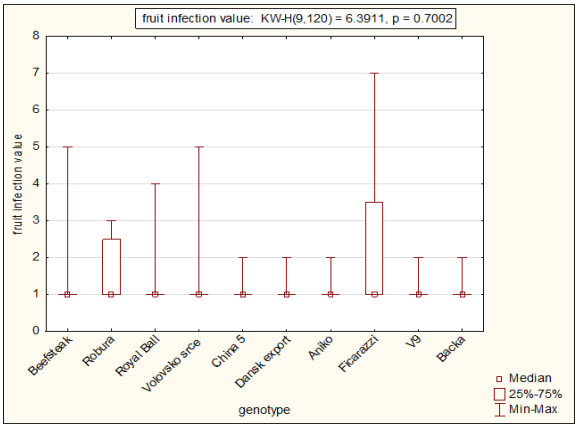

Figure 2. Intensity of early blight on the tomato fruits

Several authors (FOOLAD and LIN, 2001; FOOLAD *et al.*, 2002a; FOOLAD *et al.*, 2002b; MEDIĆ-PAP *et al.*, 2016) reported the strong correlation between EB resistance and late maturity, low yielding ability and indeterminate plant type has limited the development of lines or cultivars

with a high resistance level. Late maturing cultivars generally have an indeterminate, vine-type growth habit and continue producing new foliage throughout the season. Therefore, late maturing cultivars might appear resistant (CHAERANI and VOORRIPS, 2006). In our trial genotype Royall Ball which showed the lowest level of infection is determinate growth type and medium early cultivar. Obtained results could be explained by low infection intensity and slight insignificant differences between tested genotypes. Such level of leaves infection resulted in low fruit infection. This is in the accordance with previous research which proved that fruit infection rarely exceeded 13% in the naturally infected plants (BASU, 1974).

Biochemical parameters of the healthy and EB infected tomato fruits

The chemical composition of the fruit depends on different factors such as genetics, environmental factors, production technology and post-harvest storage conditions (BORGUINI and DA SILVA TORRES, 2009; MARŠIĆ *et al.*, 2011; VINKOVIC VRCEK *et al.*, 2011). Cultivar is a major factor contributing to the total content of phenolics in tomatoes when grown under similar environmental conditions (STEWART *et al.*, 2000). Synthesis of secondary plant metabolites could also depend on pathogen attack. Although fruits of tested tomato genotypes did not show differences in susceptibility to EB, the next step was to analyze amount of phenolic compounds and antioxidant activity in healthy and infected fruits. According to ANOVA, total phenolics (TP), tannins (TT), flavonoids (TF) and antioxidant activity in tomato fruits was significantly affected by genotype, disease occurrence and interaction of these two factors (Table 2).

Table 2 Analysis of variance for total phenolics (TP), tannins (TT), flavonoids (TF) and antioxidant activity (DPPH assay)

Trait	Source	Genotype (G)	Treatment (T)	GxT	LSD 5% (GxT)
	d.f.	9	1	9	
TP	MSS	629.39	14750.32	220.46	10,21
	F value	12.07**	282.93**	4.23**	
TT	MSS	90.67	793.67	53.93	5,89
	F value	5.23**	45.82**	3.11**	
TF	MSS	4.04	110.26	3.36	0,97
	F value	8.67**	236.26**	7.2**	
DPPH	MSS	32959	255723	71109	55,01
	F value	21.79**	169.08**	47.02**	

**Significant at 1% level; MSS=mean sum square; d.f.=degree of freedom; n=4.

The TP content in tested genotypes ranged from 45.18-76.85 for infected fruits and 22.02-50.33 mg/100g fruit weight (FW) for healthy fruits (Figure 3.). These results are in the agreement with TP content reported by MARTINEZ-VALVERDE *et al.* (2002). Clear difference is made between healthy and infected fruits. Nine genotypes showed significantly higher TP content in infected fruits (from 57.83 to 145.57%). The highest TP content was found in genotypes Beefsteak, V9 and Robura. Accumulation of phenolic compounds in the kiwifruit infected by *Botrytis cinerea* was reported by WURMS (2005).

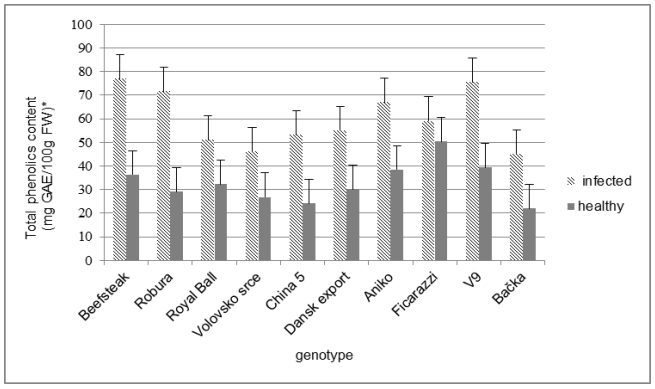

Figure 3. Total phenolics (TP) content in healthy and tomato fruits infected with *Alternaria solani*. Bars=LSD 5%

Total tannins content were ranged from 3.54 to 15.64 for healthy and 10.13-26.06 mg/100g FW for infected fruits. All tested genotypes exhibited higher content of TT in infected fruits (Figure 4.). However, this difference was significant in the five genotypes (Beefsteak, Robura, Aniko, Bačka, and China 5). In these genotypes TT content was from 1.97 to 3.75 times higher in infected fruits.

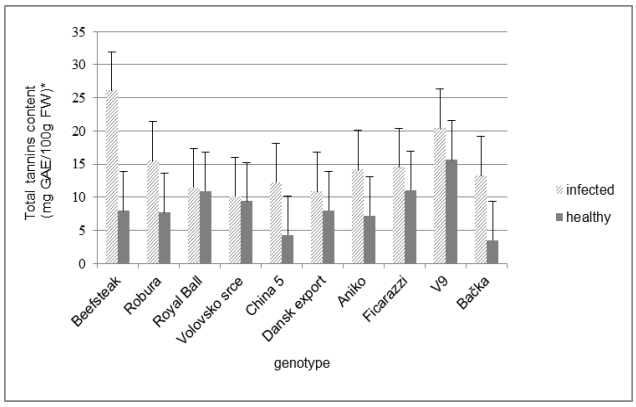

Figure 4. Total tannins (TT) content in healthy and tomato fruits infected with *Alternaria solani*. Bars=LSD

Total flavonoids content varied from 1.77 to 3.70 mg/100g FW for healthy fruits, while in the infected fruits the content of TF was from 4.05 to 6.60 mg/100g FW (Figure 5). TF content of different tomato genotypes varied from 4 to 26 mg/100g FW (SLIMESTAD *et al.*, 2008). In nine out of ten tested genotypes significant difference was found in TF content between healthy and infected fruits. Infected fruits contained 1.42 to 3.73 time's higher TF content than the healthy ones. For tomato cultivar Royal Ball, there was no significant difference in average TF content on the basis of the EB infection.

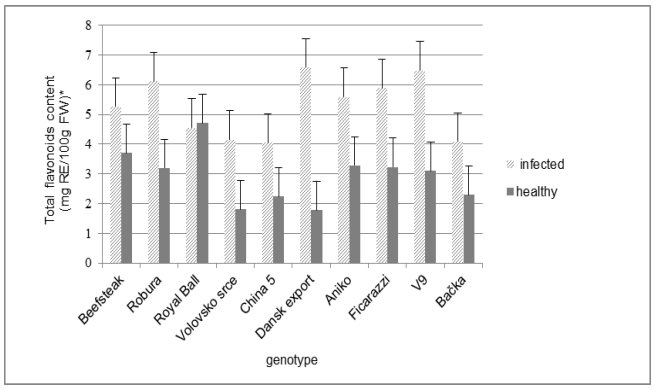

Figure 5. Total flavonoids (TF) content in healthy and tomato fruits infected with *Alternaria solani*. Bars= LSD 5%

DPPH assay stands as one of the reliable method in order to screen the plant extracts for their antioxidant potential (SANJUKTA and GHOSH, 2012). IC50 is defined as the total antioxidant necessary to decrease the initial concentration of DPPH radicals by 50%. Higher the IC50 value signifies the less antioxidant activity and vice-versa (BHOYAR *et al.*, 2011). Five out of ten tested genotypes had antioxidant activity significantly higher in infected than in healthy fruits (Figure 6). Only genotype Ficarazzi showed higher antioxidant activity in healthy fruits. The highest antioxidant activity was demonstrated by genotype Beefsteak and line V9. DPPH IC50 in infected fruits was 91.20 and 97.58 respectively, while in healthy fruits a 6.46 and 1.82 fold lower antioxidant activity was recorded. In other three tested genotypes Volovsko srce, Robura and Aniko, the DPPH IC50 were 3.79, 2.39 and 1.67 times higher in infected fruits, respectively. The differences in DPPH activity were not observed in four tested genotypes which are determinant type growth.

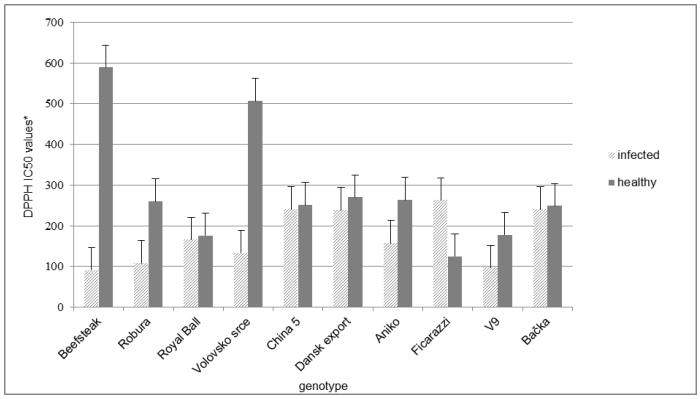

Figure 6. DPPH IC 50 activity (expressed as IC50 value (µl of sample)) in healthy and tomato fruits infected with *Alternaria solani*. Bars= LSD 5%

Significant differences for all measured parameters between healthy and infected fruits were found in three genotypes (Beefsteak, Aniko, Robura), while in two genotypes only differences between tannins were insignificant (Volovsko srce and line V9). BHATIA *et al.* (1972) reported that higher total phenolic content (tannin, flavonol and phenol) in leaves and stems correlated to EB resistance. In addition, these authors reported that the fruits of resistant varieties contained a higher amount of phenol compounds than susceptible one. According to these, results of TT, TP, TF content and antioxidant activity obtained in our trials could indicate some kind of fruit resistance of tested genotypes to EB, although the visually differences in fruits infection were not noticed.

A significant negative correlation between TP and TF with IC_{50} of DPPH radical-scavenging activity indicates that total phenolics and flavonoids in infected tomato fruits are a strong scavenger of free radicals (Table 3.). According to these data it could be concluded that tomato fruits synthesized phenolic compounds as a part of defence system. These compounds are one of the major factors in higher radical scavenging activity. Recent findings (HORSÁKOVÁ *et al*., 2013; MEDIĆ-PAP *et al*., 2014) also confirmed that antioxidant activity had increase as a result of infection. According to TERRY *et al.* (2004) fruit phenolic acids play a role in fruit defense against pathogenic attack. Concentration of phenolic acids increases in tomato fruit epicarp as part of the defence system during a pathogenic attack (RUELAS *et al.*, 2006). The results of PANE *et al.* (2011) confirmed the role of the antioxidant compounds in post-harvest tomato resistance to *Botrytis cinerea*.

Table 3. Correlation coefficients between evaluated biochemical parameters

	healthy tomato fruits				infected tomato fruits			
	DPPH[1]	TP	TT	TF	DPPH[1]	TP	TT	TF
DPPH[1]	1.00	-0,28	-0,25	-0,17	1.00	-0,52**	-0,32*	-0,17
TP		1.00	0,54**	0,41**		1.00	0,51**	0,39*
TT			1.00	0,36*			1.00	0,16
TF				1.00				1.00

[1]expressed as IC50 value (µl of sample) *Values marked with one astray are statistically significant at p>0.05 and those marked with ** are statistically significant at p>0.01

CONCLUSION

Obtained results showed some significant differences between genotypes in the intensity of symptoms on leaves, but not on the fruits. Although there were no significant differences in the intensity of symptoms on fruits, significant differences were found at biochemical level. Synthesis of phenolic compounds and antioxidant activity in EB infected fruits varied between tested genotypes. This phenomenon could be used as a one of the parameters in the evaluation of tomato fruit resistance to EB. According to these, further trials should be conducted in the conditions of artificial inoculations and between genotypes with different level of resistance.

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UTICAJ GENOTIPA PARADAJZA NA SADRŽAJ FENOLNIH JEDINJENJA I ANTIOKSIDATIVNU AKTIVOST KAO REAKCIJA PREMA PROUZROKOVAČU CRNE PEGAVOSTI

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Izvod

Crna pegavost čiji je prouzrokovač *Alternaria solani*, spada u najčešće i najdestruktivnije bolesti paradajza. Cilj ovog rada je bio da se utvrde razlike u reakciji deset genotipova paradajza (kolekcija Instituta za ratarstvo i povrtarstvo) prema crnoj pegavosti u uslovima prirodne infekcije. Ispitivani genotipovi su pokazali značajne razlike u pojavi oboljenja na listu. Intenzitet zaraženosti plodova nije se značajno razlikovao među testiranim genotipovima. Međutim, biohemijskom analizom plodova utvrđene su značajne razlike u sadržaju ukupnih fenola, tanina, flavonoida i antioksidativnoj aktivosti. Na razlike u sadržaju sekundarnih metabolita u antioksidativnoj aktivnosti uticali su genotip, zaraženost plodova i interakcija ova dva faktora. Značajne razlike između zdravih i zaraženih plodova u svim ispitivanim parametrima zabeleženi su kod genotipova Beefsteak, Aniko, Robura. Na osnovu dobijenih rezultata, sadržaj sekundarnih metabolita može se koristiti kao jedan od parametara u oceni otpornosti genotipova paradajza prema crnoj pegavosti.

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