



Biotic stress (brown rot) induces changes in enzymatic activity and antioxidant capacity in sweet cherry fruits (*Prunus avium* L.)

Boško Borković^{a*}, Đorđe Malenčić^a, Dejan Prvulović^a, Jovana Šućur^a, Mila Grahovac^a, Biljana Kiprovska^b, Ana Manojlović^a

^aUniversity of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000, Novi Sad, Serbia

^bInstitute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia

*Corresponding author: boskoborkovic@yahoo.com

ABSTRACT

Brown rot (the causal agent: *Monilinia laxa* Aderh. and Ruhl.) is one of the most common and most destructive diseases among stone fruit, sweet cherries in particular. The objective of this paper was to determine different responses to brown rot among nine sweet cherry genotypes grown under the field conditions of infection in open field. In terms of enzyme and antioxidant activity occurring under infection, the studied genotypes showed significant differences in comparison with healthy fruits. However, at the biochemical level, soluble proteins (SP), superoxide dismutase activity (SOD), guaiacol peroxidase activity (GPX), pyrogallol peroxidase activity (PPX), lipid peroxidation (LP) and antioxidant activity (ABTS and TRC assays) in sweet cherry fruits was significantly affected by genotype, plant disease and interaction of these two factors. Significant differences between studied parameters of healthy and infected fruits were recorded in genotypes Junska rana, Merchant, Sue and III/VAL. The parameters studied can be used as a method of assessment of resistance to brown rot among sweet cherry cultivars.

KEY WORDS

sweet cherry genotypes, brown rot, enzymatic activity, antioxidant capacity

Introduction

In the course of evolution, plants have evolved specific mechanisms allowing them to adapt and survive stressful conditions (Rejeb et al., 2014). Sweet cherries have been reported to contain various phenolics and anthocyanins which contribute to total antioxidant activity (Ogawa et al., 1995; Balaž et al., 2012; Wurms, 2005). Typically, diseases and pests render plants to respond with stress. Additionally, under stress conditions, tissue damage or infection, plants inherently, as a defence mechanism, induce the synthesis of polyphenolic compounds (Britton, 1983; Dixon and Paiva, 1995). *Monilinia laxa* is the predominant causal agent of brown rot among sweet cherry fruits (Balaž et al., 2012) and blossom and twig blight in stone fruit. It is believed that cracks on the cuticle, which account for 10% of fruit surface, are vital for the penetration and progress of the pathogen (Gibert et al., 2009). However, susceptibility of fruits to fruit cracking is decisive for possible infection therefore fruit rot occurs more commonly in genotypes prone to fruit cracking (Benzie and Strain, 1996). Reactive oxygen species (ROS) that involve superoxide anion radical ($\cdot\text{O}_2^-$), hydroxyl radical ($\cdot\text{OH}$) and hydrogen peroxide (H_2O_2) are considered the main sources of cell damage under biotic and abiotic stress (Mittler, 2002; Candian and Tarhan, 2003; Gara et al., 2003; Vaidyanathan et al., 2003). Within a cell, antioxidant protection is triggered in a number of cases, e.g. preventing free radicals formation and complexing, reparation of oxidation-induced damage, and promoting the elimination of the damaged molecules (Halliwell and Gutteridge, 2005). Blokhina et al. (2003) examined the levels of ROS and cell protection under stress, and highlighted the fact that plant tissues produce several enzymes and a number of non-enzymatic components of antioxidative defense system. Apel and Hirt (2004) point out that, aiming at minimizing oxidative stress, plant cells have developed a complex antioxidant system consisting of low molecular weight antioxidants as well as the enzymic antioxidant components which include superoxide dismutase, catalase, peroxidase, etc. Superoxide dismutase (SOD) belongs to a group of the most effective antioxidant enzymes which convert $\cdot\text{O}_2^-$ and H_2O_2 into H_2O and O_2 (Scandalios, 1993).

Material and methods

Plant material

Fruits of sweet cherry genotypes were collected in 2013 and 2014 from the productive orchard of the Department of Fruit growing and Viticulture on Faculty of Agriculture in Novi Sad. Fruits of nine genotypes were included in this study. The classification of the sweet cherry genotypes according to the duration of fruit ripening was determined according to the average values for 2013 and 2014. 'Burlat' matures on May 20th, and this genotype was used as a standard. Four of tested sweet cherry varieties are early ripening varieties: Burlat, Lionska rana (2 days after Burlat), Asenova rana and Junska rana (5 days after Burlat), three varieties are medium-ripening: Merchant (7 days after Burlat), Priusadebnaja and III/VAL (12 days after Burlat) and two are late-ripening: Sue (17 days after Burlat) and Summit (14 days after Burlat). Studied genotypes differ in fruit skin colour which varies from yellow to dark red. Yellow-skinned cherries with pink blush are: Priusadebnaja, Sue and Asenova rana. Red-skinned cherries are: III/VAL, Burlat, Summit, Lionska rana, Merchant, Junska rana. The trial was designed according to randomized block system in 3 replicates. Both healthy and infected fruits were sampled in the phase of botanical maturity (30 fruits from each tree). The intensity of infection of fruits in the sampling phase ranged 50–75%, while no symptoms of infection with *Monilinia laxa* were observed on healthy fruits. Fresh plant material (both healthy and infected fruits) was used for biochemical analysis. From infected fruits fragment was taken at the site of turning of healthy tissue into infected.

The content of soluble proteins and enzyme extraction

The content of soluble protein was determined by Bradford (Spector, 1978; Sedmark and Grossberg, 1997) which is based on binding of Coomassie blue G-250 to the base and aromatic amino acid residues in the protein. The reaction medium consisted of 1 mL 0.07 mol/dm³ solution Coomassie brilliant blue G-250 in 3% HClO₄ and 20 µL sample (extract of the fresh plant material). The absorbance was read after 5–30 minutes at 595 nm. The protein concentration in the sample was expressed as mg of protein per gram of fresh weight (mg protein/g FW).

Fresh fruits (1 g each) were homogenized in 10 mL of phosphate buffer (0.1 M, pH 7.0). Homogenates were centrifuged for 20 min at 10.000 x g and filtered.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed according to the method of Mandal et al. (2008) slightly modified by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) chloride. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM L-methionine, 75 µM NBT, 0.1 mM EDTA, 2 µM riboflavin and 0.02 mL of the enzyme extract. It was kept under a fluorescent lamp for 30 min, and then the absorbance was read at 560 nm. The activity of the enzyme was expressed as U per 1 mg of protein (U/mg protein).

Peroxidase (EC 1.11.1.7) activity was measured using guaiacol (guaiacol peroxidase; GPX) and pyrogallol (pyrogallol peroxidase; PPX) as substrates according to Morkunas and Gmerek (2007). Peroxidase activity (GPX and PPX) was expressed as U per 1 mg of protein (U mg/protein). PPX activity: this method includes the measurement of the content of purpurogallin - a product of pyrogallol oxidation. The enzyme extract (0.02 mL) was added to the assay mixture containing 3 mL of 180 mM pyrogallol and 0.02 mL of 2 mM H₂O₂. Absorbance was recorded at 430 nm using a spectrophotometer. GPX activity: this method consists of the assay of tetraguaiacol colored product of guaiacol oxidation in the investigated sample. The enzyme extract (0.02 mL) was added to the assay mixture containing 3 mL of 20 µM guaiacol and 0.02 mL of 3 mM H₂O₂. Absorbance was recorded at 436 nm using a spectrophotometer.

Intensity of lipid peroxidation

Lipid peroxidation (LP) was measured as malondialdehyde (MDA) equivalents production. MDA is formed through autooxidation and enzymatic degradation of polyunsaturated fatty acids in cells. This secondary end product of the oxidation of polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) via an acid-catalyzed nucleophilic-addition reaction yielding a pinkish-red chromagen with an absorbance maximum at 532 nm (Hodges et al., 1999). For this assay, plant material-fruits of cherries were first homogenized and then extracted in 10% trichloroacetic acid (TCA) in ratio 1:5 (w/v) and centrifuged at 12000 x g for 30 min at 4°C. 1 mL of supernatant was incubated with 4 mL 20% TCA containing 0.5% TBA for 30 min at 95°C. The reaction was stopped by cooling on ice for 10 min and the product was centrifuged at 10000 x g for 15 min. Lipid peroxidation (LP) was measured as nmol malondialdehyde (MDA) equivalents /g fresh weight (FW)

TRC (total reduction capacity) assay and ABTS assay

The reducing capacity of the extracts was determined by the method of Oyaizu (1986). This method is based on the ability of antioxidants to reduce Fe (III) hexacyanate to Fe (II) hexacyanate which leads to the increase in the absorbance of the reaction mixtures. This total reducing capacity (TRC) assay is based on the ability of antioxidants to react with metal ions, when reduction of Fe (III) hexacyanate to Fe (II) hexacyanate takes place. Reaction mixtures were prepared by mixing 100 μ L of the acetone extract, 2 mL of 1% solution $K_3[Fe(CN)_6]$, phosphate buffer and water. The mixtures were incubated at 50°C for 30 min, then the solution of trichloroacetic acid (10%) and $FeCl_3$ was added. Absorbance of obtained mixtures was measured at $\lambda=700$ nm; results were expressed as mg trolox equivalents per g of FW.

ABTS radical scavenging activity was performed according to the method of (Re et al., 1999) with small changes. This method is similar to DPPH assay. Antioxidant molecules react with stable ABTS radical, inhibit harmful reactions and suppress the colour development. Briefly, the ABTS stock solution was prepared from 7 mM ABTS and 2.45 mM potassium persulphate in a volume ratio of 1:1 and then incubated in the dark for 16 h at room temperature. The reaction mixture of the trial involves 3 mL of ABTS reagent and 20 μ L of an acetone extract of the sample. Absorbances were read at 734 nm. ABTS test included extracts. It was calculated based on the trolox calibration curves and was expressed as trolox per gram of fresh weight (mg of TE g/DW).

Statistical analysis

Results were expressed as mean of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by comparison of means by Fisher LSD test ($P<0.05$), using STATISTICA software for Windows version 12 (StatSoft, Inc., USA).

Results and discussion**Symptoms of brown rot**

Fruits infected with *Monilinia laxa* (Figure 1) showed signs of fruit rot. In infected fruits flesh was softer than in healthy ones. Off-white mold could be seen at sites of infection, mainly in the form of roundish 'pimples', covering them fully or partially. Freckles spreaded concentrically as the disease developed, and in conditions of higher air temperature and humidity for a several successive days, brown rot covered the entire fruit. Typically, the pathogen penetrates into cracks on fruit skin however the infection may also develop on healthy fruits by contact with infected ones (Hrustic et al., 2012). The two-year investigation showed that infected fruits either fell off and grew rotten or just shriveled, got dry and remained on branches throughout the winter. The fungus prevails in the form of mycelium in mummies or in infected twigs. Locally, conidia play a key role in developing initial infections and their spread. Apothecia and ascus with ascospore (Figure 1) rarely occur (Balaz et al., 2012). The intensity of the infection varied among investigated genotypes, but the symptoms did not differ significantly between them.



Figure 1. Symptoms of brown rot on sweet cherry fruit caused by the *Monilinia laxa* (a) and microscopic image of conidia (b)

Slika 1. Simptomi mrke truleži na plodu trešnje prouzrokovao gljivom *Monilinia laxa* (a) i mikroskopski snimak konidije (b)

Biochemical characteristics

Protein content and enzyme extraction. Fungus infection had no influence on the total protein content (Table 1) and the cherry genotypes were a significant source of variation. According to ANOVA, activity of PPX and GPX, activity of SOD and the antioxidant activity of the fruit extracts, the sweet cherry fruits were significantly affected by genotype, disease occurrence and interaction of these two factors. The results of Malenčić et al. (2010) inferred the influence of phytopathogenic fungi on plants, manifested in changes of antioxidant enzyme activity, which varied among genotypes. In infected fruits the enzymatic activity was not correlated with protein content, but in healthy fruits it was.

Table 1

Analysis of variance for Soluble protein content (SP), Activity of superoxid dismutase (SOD), guaiacol peroxidase (GPX), pirogalol peroxidase (PPX), Total reducing capacity (TRC), ABTS radical scavenging activity (ABTS)

Tabela 1

Tabela analize varijanse za sadržaj rastvorljivih proteina (SP), aktivnost superoksid dismutaze (SOD), gvajakol peroksidaze (GPX), pirogalol peroksidaze (PPX), ukupni redukcionni kapacitet (TRC), ABTS antioksidantnu aktivnost (ABTS)

Trait	Source	Genotype	Treatment	GxT
	d.f.	(G) 8	(T) 1	8
SP (Soluble protein content)	MS	54.2	1.69	82.9
	F value	55.6***	1.73	84.9***
	<i>p</i> -value	0.00	0.19	0.00
SOD (Activity of superoxid dismutase)	MS	27.0	18.4	3.0
	F value	76.1***	51.9***	8.6***
	<i>p</i> -value	0.00	0.00	0.00
GPX (Activity of guaiacol peroxidase)	MS	0.2	0.2	0.1
	F value	48.9***	54.2***	35.5***
	<i>p</i> -value	0.00	0.00	0.00
PPX (Activity of pirogalol peroxidase)	MS	0.03	0.04	0.02
	F value	46.6***	50.8***	25.5***
	<i>p</i> -value	0.00	0.00	0.00
TRC (Total reducing capacity)	MS	88.1	27.6	30.0
	F value	24,525***	7,696***	8,352***
	<i>p</i> -value	0.00	0.00	0.00
ABTS (ABTS radical scavenging activity)	MS	875.44	70.3	120.8
	F value	1,819.9***	146.2***	251.1***
	<i>p</i> -value	0.00	0.00	0.00

Note **statistically significant at $p \leq 0.05$; ***statistically significant at $p \leq 0.01$; MS=mean square; d.f.=degrees of freedom. n=3

Total protein content in fruits of investigated varieties ranged from 5.42 to 15.74 mg/g. The highest protein content was measured in infected fruit varieties III/VAL (15.74 mg/g), while the lowest protein content was measured in infected fruit varieties Lionska rana (5.42 mg/g). In genotypes Junska Rana, Priusadebnaja and Summit (Table 2) there were no statistically significant differences in total protein content measured in healthy and infected fruits.

In genotypes studied, the SOD activity ranged from 3.74 to 12.52 U/mg of protein. The lowest SOD activity was found in infected fruits of genotype Lionska Rana, and the highest in infected fruits of 'Burlat'. Overall, the increased SOD activity was higher in infected fruits, which was anticipated given that oxygen radicals triggered by this enzyme are converted into peroxides under plant stress. A few experiments performed so far have confirmed that a more intense SOD activity in plant tissue increases the tolerance of plants to environmental stress conditions (Perl et al., 1993; Allen et al., 1997). The increased value of this enzyme in infected fruits was observed in genotypes Asenova Rana, Burlat, III/VAL, Merchant, Priusadebnaja, Sue and Summit, while genotypes Junska Rana and Lionska Rana had the opposite performance. In his studies of SOD content in different ripening phases among the different fruit, Baker (1976) obtained the following values: tomato 19.6–19.8, banana 3.6–3.8, avocado 30.4–38.5 and apple 3.2–4.0 U/mg of protein.

An increased activity of guaiacol-peroxidase (GPX) occurred in fruits of genotypes Burlat and Summit after the infection, while in infected fruits of genotypes III/VAL, Merchant and Priusadebnaja no statistically significant differences in the enzyme activity was found, compared to healthy fruits. Štajner et al. (2006, 2008) found that different forms of biotic or abiotic stress increase peroxidase activity. In other cultivars examined, the activity of this enzyme decreased in the presence of *Monilinia laxa*. The lowest guaiacol-peroxidase enzyme activity was observed in fruits of genotype III/VAL, although under infection, this genotype displayed the highest protein content among the other genotypes studied. Peroxidase have an impact on the change in taste, color, texture and nutritional value of fruits (Filis et al., 1985) including changes in the process of maturation (Miesle et al., 1991).

Pyrogallol peroxidase activity (PPX) in healthy fruits varied from 0.32 to 0.53 U/mg of protein, whereas in infected ranged of from 0.21 to 0.59 U/mg protein. The lowest activity was noted in infected fruit genotype Asenova rana, while the highest activity was found in the infected fruit of 'Merchant'. Lower activity was observed in most genotype studied under infection, which may suggest increased ROS production which could not be neutralized by the plant.

The studies of Borguini and Da Silva Tores (2009) showed that the chemical composition of the fruit is governed by both genetic and environmental factors, technology of production and storage conditions. Sweet cherry genotypes respond differently to fruit rot, whereby fruits with thicker fruit skin are less sensitive to those with thinner one (Holb, 2006). Variety selection, breeding of less susceptible varieties, reduce the risk of disease incidence (Brown and Wilcox, 1989).

Table 2

Content of soluble proteins (SP), Superoxide dismutase activity (SOD), Guaiacol peroxidase activity (GPX), Pyrogallol peroxidase activity (PPX) in healthy and sweet cherry fruits infected with with *M. laxa*.

Tabela 2

Sadržaj rastvorljivih proteina (SP), aktivnost superoksid dismutaze (SOD), aktivnost gvajakol peroksidaze (GPX), aktivnost pirogalol peroksidaze (PPX) u zdravim i zaraženim plodovima sa *M. laxa*.

Genotype	status	SP,	SOD,	GPX,	PPX,
		content of soluble proteins mg/g	superoxide dismutase U/mg protein	guaiacol peroxidase U/mg protein	pyrogallol peroxidase U/mg protein
		$\bar{X} \pm Sd$	$\bar{X} \pm Sd$	$\bar{X} \pm Sd$	$\bar{X} \pm Sd$
Asenova rana	infected	8,89±1,32*	7,87±0,75	0,08±0,01*	0,21±0,01*
	healthy	6,82±0,99	7,66±0,70	0,18±0,01	0,42±0,02
Burlat	infected	8,17±0,95*	12,52±0,95	0,23±0,02*	0,37±0,06
	healthy	9,66±1,22	8,77±0,72	0,12±0,03	0,32±0,04
III/VAL	infected	15,74±1,72*	9,82±1,05	0,07±0,01	0,31±0,02*
	healthy	10,27±1,12	8,14±0,91	0,07±0,01	0,33±0,01
Junska rana	infected	10,02±1,20	7,49±0,81	0,32±0,01*	0,49±0,07
	healthy	9,96±0,82	8,36±0,92	0,46±0,04	0,43±0,06
Lionska rana	infected	5,42±0,65*	3,74±0,39	0,13±0,02*	0,36±0,05*
	healthy	12,99±1,45	4,16±0,48	0,65±0,09	0,50±0,03
Merchant	infected	12,1±1,53*	7,48±0,63*	0,37±0,01	0,59±0,02*
	healthy	8,83±1,18	5,27±0,62	0,36±0,05	0,51±0,02
Priusadebnaja	infected	8,86±0,53	5,07±0,45*	0,31±0,01	0,44±0,02*
	healthy	9,28±1,08	4,25±0,48	0,31±0,01	0,51±0,01
Sue	infected	9,64±0,72*	6,2±0,49*	0,31±0,02*	0,28±0,01*
	healthy	12,06±1,44	4,95±0,34	0,94±0,11	0,53±0,01
Summit	infected	8,64±0,92	8,47±0,71*	0,4±0,06*	0,33±0,03*
	healthy	9,19±1,06	6,54±0,57	0,17±0,02	0,35±0,04

Note *statistically significant at $p \leq 0.05$; Values are presented as means±SE for n=3. The values were subjected to ANOVA and the means were compared with the LSD test (Bars LSD 5%).

Oznaka *pokazuje statistički značajene vrednosti za $p \leq 0.05$; Vrednosti predstavljaju aritmetičke sredine za n=3. Obrada podataka izvršena je analizom varijanse (ANOVA), aritmetičke sredine su poredjene LSD testom (LSD 5%).

Activity of lipid peroxidation. Intensity degree of LP ranged from 5.52-9.17 nmol MDA/g FW in healthy and 5.76–10.76 nmol MDA/g FW in infected fruits, respectively (Figure 2). In fruits of genotypes Burlat, Merchant, Priusadebnaja and Summit infected with *Monilinia laxa*, a significant increase in intensity of lipid peroxidation was recorded. In infected fruits of genotypes Asenova rana, Junska rana and III/VAL, a decrease in intensity of lipid peroxidation was observed, whereas 'Sue' showed no statistically significant difference in LP intensity as compared to healthy fruits. Fruits of 'Summit' displayed the biggest increase in intensity of LP under infection (10.76 nmol MDA/g FW) – 50.34% more in comparison to healthy. The studies showed that the presence of *Sclerotinia sclerotiorum* fungus, originating systematically from the

same family as *Monilinia laxa* fungi, also induced the increase in LP in the soybean plant after the infection (Malenčić et al., 2010). In addition, Kiprovski et al. (2014) came up with results which suggested the increase in intensity of LP in leaves and root of sugar beet in the presence of *Rhizoctonia solani* fungus. The process of LP is considered a marker of oxidative stress in cell membranes resulting from ROS induction (Petersen et al., 1999). The majority of fruits of early ripening cultivars showed lower levels of LP in conditions of infection. Yang et al. (2008) were investigating lipid peroxidation of postharvest banana fruit during softening. Intensity of lipid peroxidation ranged from 4,64–51,78 MDA/g FW depending on the day of storage. Lower MDA values in sweet cherries may be the reason for the presence of phytochemicals (polyphenols) which act as defense ROS against.

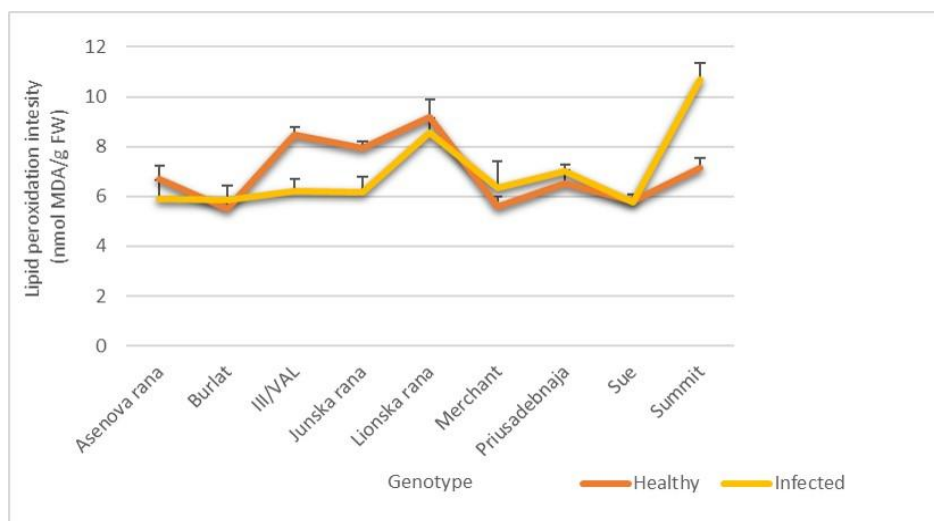


Figure 2. Changes in the lipid peroxidation intensity (LP) in healthy and sweet cherry fruits infected with *Monilinia laxa*. Values are presented as means \pm SE for n=3. The values were subjected to ANOVA and the means were compared with the LSD test (Bars LSD 5%).

Slika 2. Promene intenziteta lipidne peroksidacije (LP) u zdravim i zaraženim plodovima trešnje sa *Monilinia laxa*. Vrednosti predstavljaju aritmetičke sredine za n=3. Obrada podataka izvršena je analizom varijanse (ANOVA), aritmetičke sredine su poredjene LSD testom (LSD 5%).

TRC (total reduction capacity) assay. TRC (total reducing capacity) assay is based on the principle of increase in absorbance of reaction mixtures (Oyaizu, 2008) with increase absorbance indicating increased antioxidant activity. Trolox was used as a standard equivalent antioxidant. As indicated in Figure 3., TRC values varied from 4.14–18.58 mg TE/g FW in healthy fruits and 4.67–14.63 mg TE/g FW in infected fruits. The highest TRC activity was observed in the acetone extract of healthy fruit of genotype III/VAL (18.58 \pm 1.40 mg TE/g FW), whereas the lowest was found in the acetone extract of healthy fruit of genotype Sue (4.14 \pm 0.88 mg TE/g FW). The majority of healthy fruits showed higher TRC unlike genotypes with yellow and red fruit skin. 'Sue' and 'Priusadebnaja' that showed greater activity under infection. Liu et al. (2014) examined the antioxidant value measuring TRC assay in 110 fruits and vegetables. The values ranged from 0.08 to 21.72 mg vitamin C/g FW. Parikh and Patel, (2016) found that methanolic extract of *Manilkara hexandra* fruit with 3025.00 mg TE/100 g FW.

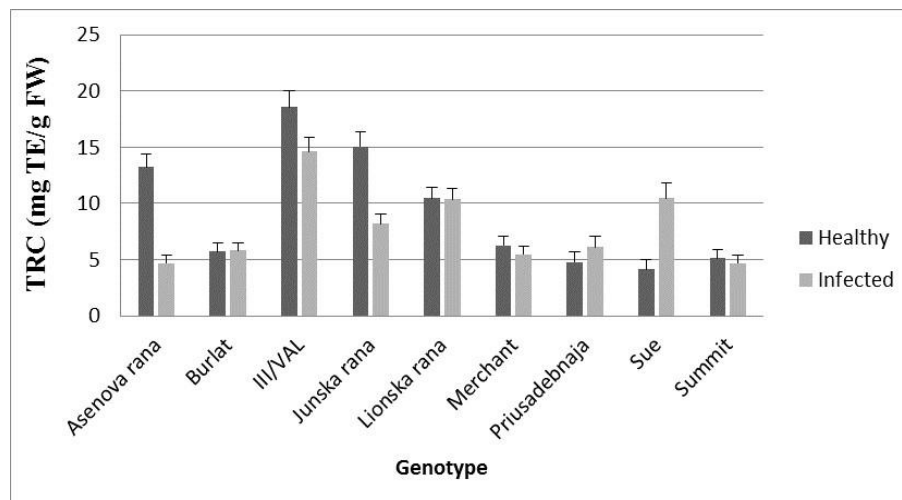


Figure 3. TRC (total reducing capacity) in healthy and sweet cherry fruits infected with *Monilinia laxa*. Values are presented as means \pm SE for n=3. The values were subjected to ANOVA and the means were compared with the LSD test (Bars LSD 5%).

Slika 3. TRC (ukupni redukcion kapacitet) u zdravim i zaraženim plodovima sa *Monilinia laxa*. Vrednosti predstavljaju aritmetičke sredine za n=3. Obrada podataka izvršena je analizom varijanse (ANOVA), aritmetičke sredine su poredjene LSD testom (LSD 5%).

In the cases of presence of anthocyanins in the plant, the DPPH method is not sufficiently precise, which is not the case with the ABTS test especially when the absorption is measured at 734 nm (Arnao, 2000). Of all fruit extracts of the genotypes studied (Figure 4), the highest antioxidant activity was found in genotype III/VAL (52.53 \pm 4.6 mg TE/g FW) whereas 'Sue' had the lowest antioxidant activity (5.56 \pm 0.5 mg TE/g FW). Under infection, genotype III/VAL exerted lower antioxidant capacity, while 'Sue' performed quite the opposite. The results inferred the following antioxidant activity of extracts in fruits of two wild cherry cultivars measured by ABTS test: cultivar B: 4.99 \pm 0.2 mg dry extract/mL and cultivar R: 48.64 \pm 0.8 mg dry extract/mL (Petković et al., 2014).

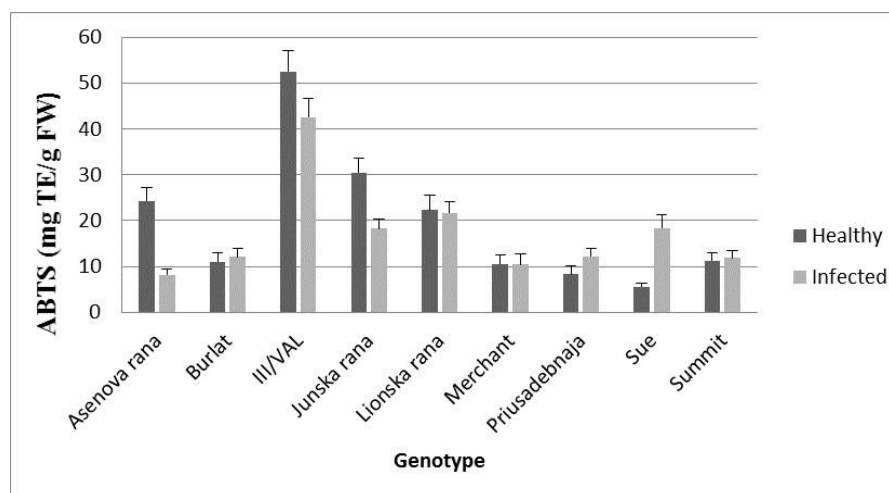


Figure 4. ABTS activity in healthy and sweet cherry fruits infected with *Monilinia laxa*. Values are presented as means \pm SE for n=3. The values were subjected to ANOVA and the means were compared with the LSD test (Bars LSD 5%).

Slika 4. ABTS aktivnost u zdravim i zaraženim plodovima trešnje sa *Monilinia laxa*. Vrednosti predstavljaju aritmetičke sredine za n=3. Obrada podataka izvršena je analizom varijanse (ANOVA), aritmetičke sredine su poredjene LSD testom (LSD 5%).

Conclusion

Sweet cherry is a rich source of various phytochemicals. Under natural infection under field conditions, the studied varieties of sweet cherries exhibited statistically significant differences in the observed parameters. Tested parameters varied among genotypes, in both healthy and infected fruits. Some genotypes showed higher antioxidant capacity and enzymatic activity under infection (genotypes with yellow-red skin—Priusadebnaja and Sue). The studied parameters can be used as a method for assessment of resistance to brown rot among sweet cherry cultivars. According to these, further trials should be conducted in the conditions of artificial inoculations and between genotypes with different level of resistance.

References

- Allen, R.D., Well, R.P., Schake, S.A. 1997. Use of transgenic plants to study antioxidant defenses. *Free. Radic. Biol. Med.* 23: 473–479.
- Apel, K., Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55: 373-399.
- Arnao, M.B. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practice case. *Tr. Food Sc. Tehnol.* 11(11): 419–421.
- Baker, J.E. 1976. Superoxide dismutase in ripening fruits. *Plant. Physiol.* 58: 644- 647.
- Balaž, J., Ognjanov, V., Iličić, R., Grahovac, M. 2012. Važnije mikoze i bakterioze trešnje. *Biljni lekar.* 40(4): 316-335.
- Benzie, I.F.F., Strain, J.J. 1996. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem.* 239: 70-76.
- Blokhina, O., Virolainen, E., Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review, *Ann. Bot.* 91: 179-194.
- Borguini, R.G, Da Silva Torres, E.A.F. 2009. Tomatoes and tomato products as dietary sources of antioxidants. *Food Rev Int.* 25(4): 313-325.
- Britton, G. 1983. *The biochemistry of natural pigments*, Cambridge University Press. Cambridge. UK.
- Brown, S.K., Wilcox, W.F. 1989. Evaluation of cherry genotypes for resistance to fruit infection by *Monilinia fructicola* (Wint.) Honey. *Hortscience.* 24: 1013–1025.
- Candan, N., Tarhan, L. 2003. The correlation between antioxidant enzyme activities and lipid peroxidation levels in *Mentha pulegium* organs grown in Ca^{2+} , Mg^{2+} , Cu^{2+} , Zn^{2+} and Mn^{2+} stress conditions. *Plant Sci.* 163: 769-779
- Dixon, R.A., Paiva, N.L. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell.* 7: 1085-1097.
- Filis, B., Sauvage, F.X., Nicolas, J. 1985. Tomato peroxidases, purification and some properties. *Sciences des Aliments* 5: 217-232.
- Gara, L.D., Pinto, M.C., Tommasi, F. 2003. The antioxidant systems vis-à-vis reactive oxygen species during plant-pathogen interaction. *Plant Physiol. Biochem.* 41: 863-870.
- Gibert, C., Chadoeuf, J., Nicot, P., Vercambre, G., Genard, M., Lescourret, F. 2009. Modelling the effect of cuticular crack surface area and inoculum density on the probability of nectarine fruit infection by *Monilinia laxa*. *Plant. Pathol.* 58: 1021-1031.
- Halliwell B, Gutteridge, J. M. C. 2005. *Free Radicals in Biology and Medicine*. Oxford. UK: Oxford University Press.
- Hodges, D.M., DeLong, J.M, Forney, C.F., Prange, R.K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 207: 604–611.
- Holb, I.J. 2006. Possibilities of brown rot management in organic stone fruit production in Hungary. *Int. J. Hortic. Sci.* 12: 87-91.
- Hrustić, J., Mihajlović, M., Grahovac, M., Delibašić, G., Bulajić, A., Krstić, B., Tanović, B. 2012. Genus *Monilinia* on pome and stone fruit species. *Pestic. Phytomed.* 27(4): 283–97.
- Kim, D.O., Heo, H.J., Kim Y.J., Yang, H.S., Lee, C.Y. 2005. Sweet and Sour Cherry Phenolics and Their Protective Effects on Neuronal Cells. *J. Agric. Food. Chem.* 53(26): 9921-9927.
- Kiprovski, B., Malenčić, Đ., Popović, M., Stojšin, V., Budakov, D., Čurčić, Ž., Danojević, D. (2014): Correlation between lipid peroxidation and phenolics content in leaves and roots of sugar beet infected with *Rhizoctonia solani*. *Phytoparasitica.* 42: 199-203.
- Liu, C., Zhao, Y., Li, X., Jia, J., Chen, Y., Hua, Z. 2014. Antioxidant capacities and main reducing substance contents in 110 fruits and vegetables eaten in China. *Food Nutr. Sci.* 5: 293–307.
- Malenčić, Đ., Kiprovski, B., Popović, M. T., Prvulović, D., Miladinovic, J. A., Đorđević, V.B. 2010. Changes in antioxidant systems in soybean as affected by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant. physiol. and biochem.* 48: 903-908.
- Mandal, S., Mitra, A., Mallick, N. 2008. Biochemical characterization of oxidative burst during interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. sp. *lycopersici*. *Physiol. Mol. Plant. P.* 72: 56–61.

- Miesle, T.J., Proctor, A., Lagrimini, L.M. 1991. Peroxidase activity, isoenzymes, and tissue localization in developing highbush blueberry fruit. *J. Am. Soc. Hortic. Sci.* 115: 827-830.
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trend. Plant. Sci.* 7: 405- 410.
- Morkunas, I., Gmerek, J. 2007. The possible involvement of peroxidase in defence of yellow lupine embryo axes against *Fusarium oxysporum*. *J. Plant Physiol.* 164: 185-194.
- Ogawa, J.M., Zehr, E. I., Bird, W. 1995. Brown rot. In: Eds. J.M. Ogawa, E. I. Zehr, G. W. Bird, D. F. Ritchie, K. Uriu and J. K. Uyemoto). *Compendium of Stone Fruit Diseases*. American Phytopathological Society, St. Paul, MN.
- Oyaizu, M. 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* 44: 307–315.
- Perl A., Perl-Treves. R., Galili, S., Aviv, D., Shalgi, E., Malkin, S., Galun, E. 1993. Enhanced oxidative-stress defense in transgenic potato overexpressing tomato Cu, Zn superoxide dismutase. *Theor. Appl. Genet.* 85: 568–576.
- Petersen D.R., Reichard, J., Kolaja, K. L., Hartley, D. 1999. 4-Hydroxynonenal and malondialdehyde hepatic protein adducts in rats treated with carbon tetrachloride: immunochemical detection and lobular localization. *Toxicol. Appl. Pharm.* 161: 23–33.
- Petković, B., Matoš, S., Gorgi, N., Kukrić, Z. 2014. Analysis of antioxidant activity of different species of wild cherry *Prunus avium* L. *Glo. Adv. Res. J. Agric. Sci.* 3(5): 128-135.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26: 1231-1237.
- Rejeb, I.B., Pastor, V., Mauch-Mani, B. 2014. Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms. *Plants* 3: 458-475.
- Scandalios, J.G. 1993. Oxygen stress and superoxide dismutases. *Plan. Physiol.* 101: 7-12.
- Sedmark, J., Grossberg, S.E. 1977. A rapid, sensitive and versatile assay for protein using Coomassie Brilliant Blue G250. *Anal Biochem.* 79: 544–552.
- Spector, T. 1978. Refinement of the Coomassie blue method of protein quantitation. *Anal Biochem.* 86: 142–146.
- Štajner, D., Milić, N., Čanadanović-Brunet, J., Kapor, A., Štajner, M., Popović, B.M. 2006. Exploring *Allium* species as a source of potential medicinal agents. *Phytother. Res.* 20: 581-584.
- Štajner, D.I., Popović, B. M., Čanadanović-Brunet, J. M., Štajner, M. 2008. Antioxidant and scavenger activities of *Allium ursinum*. *Fitoterapia.* 4: 303-305.
- Yang, Z., Su, X., Prasad, K. N., Yang, B., Cheng, G., Chen, Y., Yang, E., Jiang, Y. 2008. Oxidation and peroxidation of postharvest banana fruit during softening. *Pak. J. Bot.* 40(5): 2023-2029.
- Vaidyanathan, H., Sivakumar, P., Chakrabarsty, R., Thomas, G. 2003. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.)-differential response in salt-tolerant and sensitive varieties. *Plant Sci.* 165, 1411-1418.
- Wurms, K.V. 2005. Susceptibility to *Botrytis cinerea*, and curing induced responses to lytic enzymes and phenolics in fruit of two kiwifruit (*Actinidia*) cultivars. *N. Z. J. Crop. Hort.* 33: 25-35.

Promene u enzimatskoj aktivnosti i antioksidantnom kapacitetu plodova trešnje (*Prunus avium* L.) izazvane biotičkim stresom

Boško Borković^{a*}, Đorđe Malenčić^a, Dejan Prvulović^a, Jovana Šučur^a, Mila Grahovac^a, Biljana Kiprovska^b, Ana Manojlović^a

^aUniverzitet u Novom Sadu, Poljoprivredni fakultet, Trg Dositeja Obradovića 8, 21000, Novi Sad, Srbija

^bInstitut za ratarstvo i povrtarstvo, Maksima Gorkog 30, 21000, Novi Sad, Srbija

^{a*}Autor za kontakt: boskoborkovic@yahoo.com

SAŽETAK

Mrka trulež (uzročnik: *Monilinia laxa* Aderh. i Ruhl.) je jedna od najčešćih i najdestruktivnijih bolesti koštičavih voćaka, naročito trešnje. Cilj ovog rada je bio da se utvrde različiti odgovori na mrku trulež ploda između devet genotipova trešnje, uzgajanih u uslovima prirodne infekcije na otvorenom polju. U pogledu enzimatske i antioksidativne aktivnosti koja se javila u uslovima infekcije, ispitivani genotipovi su pokazali značajne razlike u odnosu na zdrave plodove. Na razlike u biohemijskom pogledu uticali su: genotip, prisustvo biljne bolesti kao i interakcija ova dva faktora. Biohemijski parametri koji su pokazali razlike u uslovima infekcije su: sadržaj rastvorljivih proteina (SP), aktivnost supereksid dismutaze (SOD), aktivnost gvajakol peroksidaze (GPX), aktivnost pirogalol peroksidaze (PPX), lipidna peroksidacija (LP) i antioksidativna aktivnost (ABTS i TRC testovi). Značajne razlike između ispitivanih parametara zdravih i zaraženih plodova zabeležene su u genotipovima Junska rana, Merchant, Sue i III/VAL. Ispitani parametri se mogu koristiti kao metoda procene otpornosti na mrku trulež između genotipova trešnje.

KLJUČNE REČI

genotipovi trešnje, mrka trulež, enzimaska aktivnost, antioksidanti kapacitet

Primljen: 28.07.2017.

Prihvaćen: 05.12.2017.