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## Antioxidant response of sweet pepper fruits infected with *Alternaria alternata*

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**Abstract:** *Capsicum annuum* is valuable source of many bioactive compounds with the protective role in plants against biotic and abiotic stress as well as beneficial effect on humans' health. This vegetable is susceptible to many infections, including postharvest decay caused by fungus *Alternaria alternata*. In order to better understanding pepper fruits defense system, the concentration of phenols and ascorbic acid, scavenging activity and antioxidant enzyme activity in three kapia type sweet pepper fruits (Amfora, Una and Kurtovka kapia) infected with fungus *A. alternata* were determined in this study. Amfora fruits had the highest tolerance to *Alternaria* infection. Amfora and Una increase total phenol and vitamin C content after wounding and inoculation, while Kurtovska kapia decreased amount of vitamin C. Depending on reaction mechanism, antioxidant tests showed no changes or decrease in antioxidant capacity in treated fruits. Except for phenylalanine ammonia-lyase activity in Amfora and Kurtovska kapia and ascorbate peroxidase activity in wounded Kurtovska kapia fruits, all measured enzyme activity showed no changes or decrease by wounding and/or *Alternaria* infection. According to results of intensity of lipid peroxidation as biological marker of oxidative stress, it can be concluded that wounding and infection disturb redox balance in all examined genotypes. The tested genotypes showed certain difference in antioxidant defence against wounding and pathogen stress.

**Keywords:** *Capsicum annuum*; non-enzymatic antioxidants; *Alternaria* infection; oxidative stress; antioxidant enzymes.

### INTRODUCTION

Pepper (*Capsicum annuum* L.) is a member of *Solanaceae* family<sup>1</sup> and globally adopted important horticultural crop. Besides their nutritional value, *Capsicum* fruits have health-protective effect. They are a significant source of min-

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erals, vitamins, carotenoids, capsaicinoids and phenolic compounds with a beneficial effect on human health due to their antioxidant properties as well as ability to protect cells against damages caused by oxidative stress and prevent development of illness such as cardiovascular diseases, Parkinson's, Alzheimer's, cancer and diabetes.<sup>2</sup>

On the other side, many abiotic and biotic stress factors affect the production of *Capsicum* species, including a great number of fungi. The following fungal species: *Fusarium solani*, *Fusarium subglutinans*, *Alternaria alternata*, *Alternaria tenuis*, *Botrytis cinerea*, *Colletotrichum* species complex and *Verticillium* spp are the common causal agents of pepper fruit rots.<sup>3</sup> The numerous *Alternaria* species are known as widespread plant pathogens of various types of horticultural crops, responsible for significant economic losses. *A. alternata* is the main causal agent of diseases of different vegetable species including internal mold in *Capsicum annum*.<sup>4</sup>

In plants, pathogen infections provoke the production of reactive oxygen species (ROS) as one of the earliest responses in pathogen-plant interaction. ROS are by-product of aerobic metabolism and have a role as a messenger in signal transduction, regulation of metabolism, or memory formation *via* DNA methylation.<sup>5</sup> The main ROS in cells includes free radicals such as superoxide anion ( $\text{O}_2^-$ ), hydroxyl radical ( $\text{OH}^\bullet$ ), and molecular non-radical forms – singlet oxygen ( $^1\text{O}_2$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). ROS overproduction under unfavourable conditions: extreme temperatures, heavy metals, drought, salinity (abiotic stress) or pathogen infection (biotic stress), lead to oxidative stress and cause damages to major macromolecules, *i.e.*, proteins, lipids and nucleic acid.<sup>6</sup> In order to manage cascades of uncontrolled oxidation and protect plant cells from oxidative damage, plants possess potent non-enzymatic (phenolic compounds, tocopherols, carotenoids, glutathione (GSH), ascorbic acid (AA)) and enzymatic antioxidant defense system (superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR)).<sup>7</sup>

Knowledge of the relationship between the level of bioactive compounds and the antioxidant activity of sweet pepper could lead to a better understanding of pepper fruits defense system against infection. The aim of our study was to determine the concentration of phenolic compounds and ascorbic acid, scavenging activity as well as antioxidant enzyme activity in three different genotypes of kapia type sweet pepper fruits infected with fungus *A. alternata*.

## EXPERIMENTAL

### *Collection of plant material and inoculation procedure*

As plant material in this study, fruits of three different sweet peppers genotypes, Amfora, Una and Kurtovska kapia was used. The fruits were grown in the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia, in 2019 and harvested on the 16<sup>th</sup> of

October at technological maturity. Typical fruits without visible injury were selected and kept in cold storage during the night. Pericarp thickness, fruit length, width and weight were measured on 21 fruits from each genotype.

Nine selected fruits of each genotype for the disease assessment were divided into three groups: the intact fruits (control group); the fruits injected with sterile water and fruits inoculated with a fungal spore suspension. The inoculation was done following the procedure described by Fallik *et al.*<sup>8</sup> For the inoculation monohyphal isolate (K-93) of *A. alternata*, originated from infected pepper fruit was used. The isolate was identified by polymerase chain reaction (PCR) method and preliminary pathogenicity test was done before the experiment. The spore suspension ( $10^5$  conidia/ml) of the 10 days isolate grown on potato–dextrose–agar at 20 °C was used in inoculation process. Six fruits of each genotype were wounded at 3 points and inoculated by pipetting 40 µl sterile water (3 fruits) or spore suspension in each puncture (3 fruits). Intact fruits were used as a control. The fruits were put into PVC bags (due to obtain high humidity) and incubated at 20 °C for 10 days. The fruit assessment was done 10 days after inoculation. In order to evaluate the rate of the severity of fungal infection on pepper fruits, internal and external lesion diameters as well as mycelium were measured by vernier caliper.<sup>9</sup> Also, the surface area of lesions and mycelia were calculated according to the formula for the area of a circle. After disease assessment fruits were cut into small pieces and stored at –70 °C until the biochemical analyses were performed.

#### Sample extraction

Total reduction capacity (TRC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitroblue tetrazolium (NBT) assays were performed and total phenolic content was determined in methanol extracts of fruits. 4 g plant material was homogenized with 10 ml of 70 % aqueous methanol solution and after 24 h centrifuged at 5000 rpm for 15 min. Separated supernatant was kept in cold storage. PAL, SOD, CAT, APX, GPX and propoxyphene (PPX) activity assays and lipid peroxidation (LP) were measured in phosphate buffer extract prepared by extraction 2 g plant material with 10 ml 0.1 M phosphate buffer (pH 7.0). After the centrifugation (5000 rpm) at 4 °C for 15 min, supernatant was separated and kept in cold storage.

#### Biochemical assays

Folin–Ciocalteu method was used to evaluate the total phenolic content following the procedure described by Wootton-Beard *et al.*<sup>10</sup> with slight modification. The results were expressed as quercetin equivalents in mg per 100 g of fresh weight (mg QE (100 g FW)<sup>-1</sup>). The level of vitamin C in sweet pepper samples was estimated by spectrophotometric measurements.<sup>11</sup> Extracts for this assay were prepared by weighing 1 g of sweet pepper fruits, homogenized with 10 ml 5 % metaphosphoric acid + 10 % acetic acid solution. The supernatant was used for the assay. The content of vitamin C was expressed as ascorbic acid equivalents in mg per 100 g of fresh weight (mg AA (100 g FW)<sup>-1</sup>).

#### Antioxidant activity

DPPH assay was carried out by the method based on reaction between stable DPPH radical and a substance that can donate a hydrogen atom as described by Floegel *et al.*<sup>12</sup> TRC was obtained as Govindan and Muthukrishnan<sup>13</sup> previously described with some modifications. The results of antioxidant activity estimated by DPPH and TRC were expressed as mg of Trolox equivalents per 100 g of fresh weight (mg Trolox (100 g FW)<sup>-1</sup>). The methanol extract was used to estimate superoxide radical scavenging activity, while phosphate buffer was used to determine superoxide dismutase (SOD, EC 1.15.1.1) enzyme activity, while both assays were performed following the same procedure.<sup>14</sup> The results of superoxide radical scavenging

activity were expressed as the percentage inhibition of superoxide anion generation. SOD enzyme activity was expressed as units per mg of protein ( $U (mg \text{ protein})^{-1}$ ). A Bradford<sup>15</sup> method was used to quantify the total protein content and for the construction of standard curve different concentrations of albumin were taken. The total protein content was reported as protein equivalents in mg per g of fresh weight ( $mg \text{ protein (g FW)}^{-1}$ ).

#### *Antioxidant enzyme assays*

Catalase enzyme activity (CAT; EC 1.11.1.6) was determined according to the method of Aebi,<sup>16</sup> based on decreasing absorbance measured at 240 nm due to the dismutation of  $H_2O_2$ . The results were reported as units per mg of protein ( $U (mg \text{ protein})^{-1}$ ) where one unit of CAT activity was defined as the amount of enzyme that caused the composition of one  $\mu\text{mol}$  of  $H_2O_2$  per min at 25 °C. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed according to the protocol of Nakano and Asada.<sup>17</sup> A decrease in absorbance was recorded at 290 nm for 5 min and the APX activity was expressed as units per mg of protein ( $U (mg \text{ protein})^{-1}$ ). For the measurement of guaiacol (GPX; EC 1.11.1.7) and pyrogallol peroxidase (PPX; EC 1.11.1.7) activity, Morkunas and Gmerek<sup>18</sup> methods were used, and substrate oxidation was followed by the decrease in the absorbance at 470 and 420, respectively. The GPX and PPX activities were expressed as units per mg of protein ( $U (mg \text{ protein})^{-1}$ ). A protocol of Gerasimova *et al.*<sup>19</sup> was used to determine phenylalanine ammonia-lyase (PAL; EC 4.3.1.5). The PAL activity was expressed as *trans*-cinnamic acid equivalents in mg per 100 g of fresh weight ( $mg \text{ tCA (100 g FW)}^{-1}$ ).

#### *Lipid peroxidation*

The intensity of lipid peroxidation was assayed by the protocol of Heath and Packer.<sup>20</sup> The level of LP was expressed as  $\mu\text{mol}$  malondialdehyde (MDA) equivalents per mg protein ( $\mu\text{mol MDA (mg protein)}^{-1}$ ).

#### *Statistical analysis*

The data were analyzed using TIBCO Data Science-Statistica software. The normality of obtained values was tested using Shapiro–Wilk (SW) test. Given that values of diameters and area of mycelia as well as lesion did not show normal distribution, for comparing samples non-parametric Kruskal–Wallis (KW) test and Mann–Whitney U test were used. Values of morphometric parameters and biochemical assays were tested by ANOVA. In the case of biochemical assays, a comparison of means was done by the Bonferroni *post hoc* test ( $p < 0.05$ ), while for morphometric parameters was used Fisher least significant difference (*LSD*) *post hoc* test ( $p < 0.05$ ). All the evaluated parameters and their importance in the fruit–pathogen interaction was investigated by the principal component analysis (PCA).

On the graphs, results are expressed as the mean  $\pm$  standard deviation of three independent samples. Different letters within one genotype represent statistically significant differences between treatments ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

All tested genotypes have elongated fruits and intensively red at physiological maturity. Amfora and Kurtovska kapia have dark green fruits, whereas the colour of Una fruits is yellow-green at technological maturity. Measured morphometric characteristics of fruits are given in Table I.

TABLE I. Morphometric characteristics of genotypes; values preceded by the same letter in the vertical do not differ significantly according to the test of Fisher *LSD* ( $p < 0.05$ )

Genotype	Pericarp thickness, mm	Fruit length, cm	Fruit width, cm	Fruit weight, g
Amfora	4.98 <sup>a</sup>	13.30 <sup>b</sup>	6.38 <sup>a</sup>	117.33 <sup>a</sup>
Una	5.09 <sup>a</sup>	14.69 <sup>a</sup>	4.90 <sup>b</sup>	91.74 <sup>b</sup>
Kurtovska kapia	4.14 <sup>b</sup>	12.91 <sup>b</sup>	4.82 <sup>b</sup>	63.73 <sup>c</sup>

Significant differences were observed among genotypes in morphometric characteristics. According to ANOVA Amfora and Una have higher values of pericarp thickness, compared to Kurtovska kapia.

The measured values of lesions and mycelium developed on pepper fruits as a consequence of fungal infection are presented in Table II. Lesions formed on the surface of the Una genotype had the largest size. Compared with the other two tested genotypes, Amfora had ten times smaller external and internal lesions. According to the Mann-Whitney U test, the difference between lesion sizes of Una and Kurtovska kapia was not significant. On the other side, the mycelium size of all tested genotypes did not show a significant difference by KW test. This probably means that mycelium stopped growing, but fungal compounds still spread within the plant tissue. On intact fruits and sterile water injected fruits no lesions development was observed.

TABLE II. The rate of *A. alternata* infection on pepper fruits, values preceded of same letter in the vertical do not differ significantly according to the tests of Kruskal–Wallis and Mann–Whitney U ( $p < 0.05$ )

Genotype	Lesion				Mycelium			
	Diameter, mm		Area, mm <sup>2</sup>		Diameter, mm		Area, mm <sup>2</sup>	
	External	Internal	External	Internal	External	Internal	External	Internal
Amfora	1.43 <sup>a</sup>	2.39 <sup>a</sup>	6.22 <sup>a</sup>	10.89 <sup>a</sup>	1.38 <sup>a</sup>	13.44 <sup>a</sup>	1.76 <sup>a</sup>	6.11 <sup>a</sup>
Una	10.27 <sup>b</sup>	11.20 <sup>b</sup>	111.44 <sup>b</sup>	129.33 <sup>b</sup>	0.77 <sup>a</sup>	4.11 <sup>a</sup>	3.32 <sup>a</sup>	19.22 <sup>a</sup>
Kurtovska kapia	5.47 <sup>b</sup>	6.94 <sup>b</sup>	27.67 <sup>b</sup>	43.22 <sup>b</sup>	0.62 <sup>a</sup>	1.86 <sup>a</sup>	1.33 <sup>a</sup>	7.22 <sup>a</sup>

Differences in lesions diameter between tested genotypes suggested that there are probably differences in the biochemical response. Focusing on the biochemistry of the plant defence system, it had been reported that the host-pathogen interaction leads to changes in primary and secondary plant metabolites, as well as the enzyme profile as a result of excessive ROS production during host colonization.<sup>21</sup>

In the present study, ANOVA indicated that genotype, treatment, and their interaction had a significant influence (at 5 % level) on measured bioactive compounds and antioxidant capacity in fruits (*F* values are given in the Supplementary material to this paper; Table S-I). Results for measured biochemical parameters were presented in Figs. 1–5.

Obtained results demonstrated an increase in TP content in Amfora and Una in case of fungus infection (Fig. 1). This may indicate that these genotypes biosynthesize phenolic compounds as a part of the defence system against *Alternaria* infection. In the previous studies it was affirmed that the pathogen inoculation and mechanical damages provoke the accumulation of phenols.<sup>22,23</sup> The production of phytoalexins, one of the phenolic compounds toxic to many pathogens, is a systemic early response to presence of fungus and bacteria.<sup>24</sup> On the other hand, the non-existence of difference in TP content in Kurtovska kapia genotypes can suggest that this antioxidant response is genotype dependent, which is in agreement with Ribes-Moya *et al.*<sup>25</sup> Antioxidant capacity measured by DPPH, NBT and TRC showed variability depending on genotype and treatment (Fig. 1).

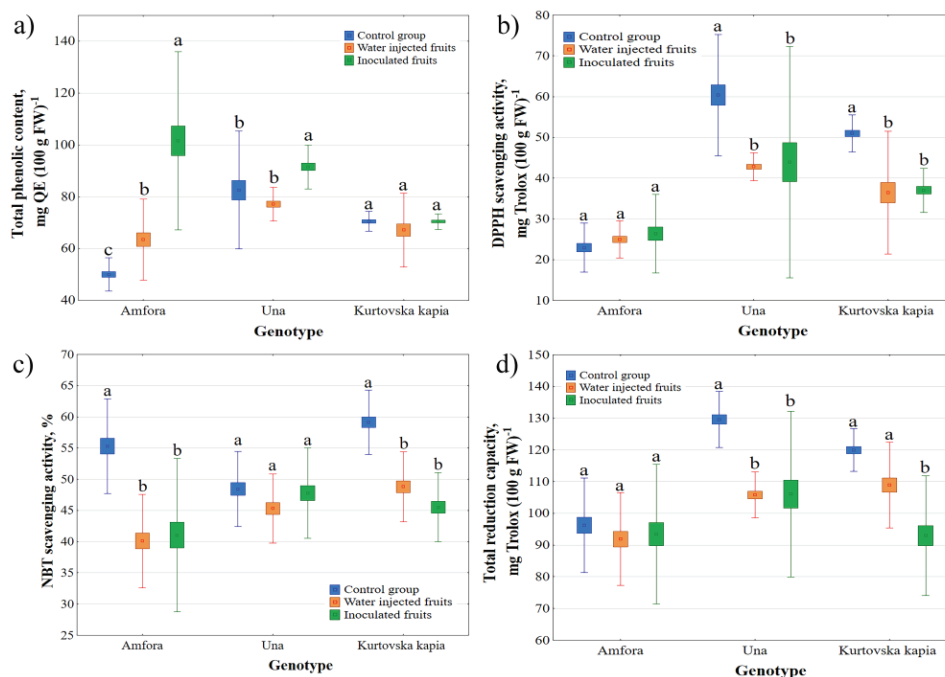


Fig. 1. Changes in a) Total phenolic content (TP) and antioxidant capacity measured by: b) DPPH; c) NBT; d) total reduction capacity (TRC).

Based on DPPH and TRC test results, in the Amfora significant changes among experimental groups were not observed. Contrary, NBT test showed decrease in antioxidant activity when treatments were applied. The opposite trend in antioxidant activity was noticed in treated fruits of Una. In Kurtovska kapia, all antioxidant tests showed decrease in antioxidant activity in infected fruits. Rubio-Melgarejo *et al.*<sup>24</sup> suggested that pathogen may manipulate the defense res-

ponse, modifying the signalling network and cause decrease in the antioxidant activity. Variations in the results of antioxidant activity may be related to the different reaction mechanisms of applied tests and the nature of the antioxidant compounds present in the sample.<sup>26</sup>

Vitamin C or ascorbic acid (AA) is one of the substances with the potential scavenging activity on pathogen-induced excessive ROS molecules. Variation in vitamin C content is related to genotype, maturity stage and agro-climatic conditions Žurawik *et al.*<sup>27</sup> The present work confirmed variations in amount of vitamin C content related to genotypes and treatment (Fig. 2). In the present study, the reduction in vitamin C level in inoculated pepper fruits of Kurtovska kapia is in accordance with findings of Tripathi and Mishra.<sup>28</sup> This fact may be associated with the oxidation of vitamin C by some degenerating oxidases produced during pathogenesis<sup>29</sup> or because of increasing rate of respiratory activity in infected tissue.<sup>30</sup> On the other hand, in Amfora and Una, vitamin C content was significantly increased by a fungal infection. Fujiwara *et al.*<sup>31</sup> also described that total AA accumulates after viral infection as well as wounding stress.

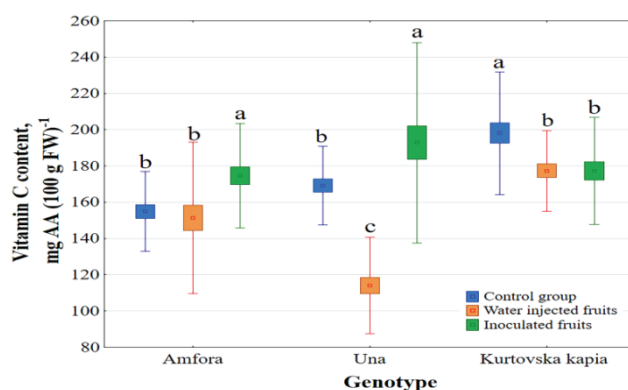


Fig. 2. Changes in vitamin C content assayed in three different genotype pepper fruits in control, water injected and inoculated groups.

ANOVA results obtained for enzymatic activity indicated that it is affected by genotype, type of treatment and the interaction of these factors. The influence of genotype, group of fruits treatment and their interaction significantly affected measured enzymatic activity at the level of 5 % (Supplementary material; Table S-II). The results of the tested enzymes are presented in Fig. 3.

CAT, SOD and peroxidases are the ROS-detoxifying enzymes which convert superoxide radicals and hydrogen peroxide into less toxic and more stable components. As a first line defence, SOD catalyses the dismutation of superoxide anion into oxygen and hydrogen peroxide, which is further catalysed by peroxidases and CAT. Peroxidases are members of a large family of enzymes, involved

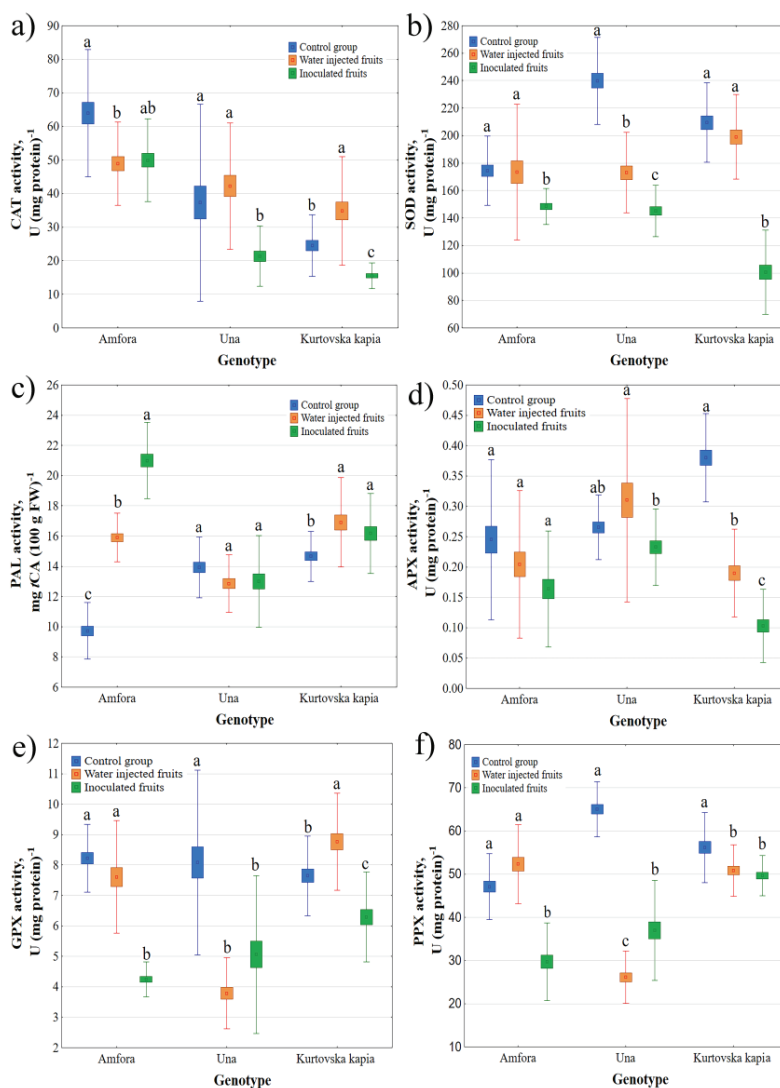


Fig. 3. Changes in: a) CAT; b) SOD; c) PAL; d) APX; e) GPX; f) PPX activity assayed in three different genotype pepper fruits in control, water injected and inoculated groups.

in hydrogen peroxide removal. The main difference between them is the type of a reducing substrate they use: ascorbate peroxidase uses ascorbate, guaiacol peroxidase guaiacol and pirogallol peroxidase pyrogallol.<sup>32</sup> Increases in CAT, SOD and peroxidase activity indicates that initial infection induced their activities and consequently the activation of antioxidant plant defense.<sup>33</sup> The activity of CAT was lower in inoculated fruits of Una and Kurtovska kapia than in control fruits. This is consistent with the results of Li *et al.*<sup>34</sup> who found decrease in CAT act-



ivity in mango fruits infected by *A. alternata*. However, water-injected fruits of Kurtovska kapia showed the highest CAT activity. In Amfora, CAT activity was significantly reduced in fruits injected with water, compared to the control group. Wounding stress in avocado fruit lead to decrease in CAT and SOD levels.<sup>35</sup> Obtained results showed that in Una water-injected fruits had significantly lower SOD activity than the control group, but higher than infected fruits. In Amfora and Kurtovska kapia significant differences were not observed when fruits were just wounded and water-injected, SOD activity in all tested genotypes was reduced by *A. alternata* infection. Wang *et al.*<sup>36</sup> reported decrease in CAT, SOD and APX in the citrus fruit infected by *Penicillium digitatum*. In contrast, *Alternaria tenuis* and *Botrytis cinerea* in peach fruits increased the activities of CAT, SOD and peroxidase in comparison to the sterile water treatments.<sup>37</sup>

GPX and PPX activity in pepper fruits was the lowest in inoculated fruits in Kurtovska kapia and Amfora, while in Una they were reduced compared to the control, being the lowest in water injected fruits. By the contrast, APX enzymatic activity was reduced only in Kurtovska kapia inoculated fruits. Borković *et al.*<sup>38</sup> also reported decrease in GPX and PPX in some genotypes of sweet cherry fruits infected by *Monilia laxa*. PAL catalyses the deamination of phenylalanine to form trans-cinnamic acid in the phenylpropanoid pathway. Cinnamic acid has role in biosynthesis of hydroxycinnamic acid derivatives like coumaric, caffeic, ferulic acid as well as flavonoids, lignins, stilbenes, tannins and anthocyanins.<sup>39</sup> In Amfora and Kurtovska kapia significant increase in PAL activity was observed both in water-injected as well as *A. alternata* inoculated fruits. Additionally, in Amfora increase in PAL activity in inoculated and wounded fruits may be related to the production of phenolic compounds as defense responses in fruits.

The level of lipid peroxidation as an indicator of ROS-induced damage under stress conditions is based on the reaction of malondialdehyde (MDA), an end-product of lipid peroxidation, with thiobarbituric acid (TBA).<sup>40</sup> Accumulation of ROS is known to increase lipid peroxidation in biological membranes due to its ability to react with the double bond of lipid hydrocarbon chains. The 1,4-pentadiene structure of a polyunsaturated fatty acid (PUFA), either free or esterified to cholesterol or glycerol, is easily oxidized by unbalanced ROS.<sup>41</sup> The level of lipid peroxidation was higher in fruits injected with water than in the control group, but the highest level of lipid peroxidation was found in the inoculated fruits (Fig. 4). Obtained results are in agreement with those of Li *et al.*<sup>34</sup> who reported an increase in MDA concentration in mango fruits infected by *A. alternata*.

To observe the relationship among examined bioactive compounds, enzyme activity, genotypes and wounding and infection stress, principal component analysis (PCA), Fig. 5, was applied. The PCA score plot (Fig. 5a) was used for studying the classification of the categorized data, while the PCA loadings plot was

used for obtaining information on the relative importance of the variables to each principal component (Fig. 5b).

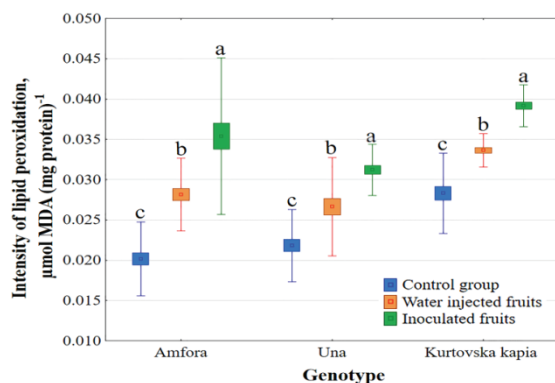


Fig. 4. Intensity of lipid peroxidation in healthy, wounded and sterile water injected as well as infected fruits in all three genotypes.

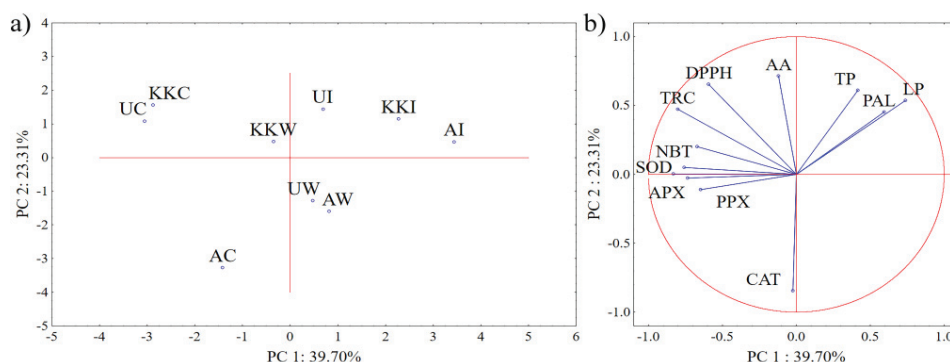


Fig. 5. Biplot of the first two principal components of principal component analysis; a) score plot of genotypes and treatment, Amfora control, water-injected, inoculated (AC, AW, AI), Una control, water-injected, inoculated (UC, UW, UI), Kurtovska kapia control, water-injected, inoculated (KKC, KKW, KKI); b) loadings plot of all evaluated biochemical parameters; total polyphenol content (TP); total ascorbic acid (AA); 2,2-dyphenyl-1-picrylhydrazyl assay (DPPH test); total reduction capacity (TRC); nitroblue tetrazolium test (NBT test); superoxide dismutase activity (SOD); intensity of lipid peroxidation (LP); phenylalanine ammonia-lyase activity (PAL); catalase activity (CAT); ascorbate peroxidase activity (APX); guaiacol peroxidase activity (GPX); pyrogallol peroxidase activity (PPX).

The first two principal components extracted by PCA covered 63.01 % of the data variance. The first component (PC1) accounted for 39.70 % of the total variance and it is highly likely that this dimension separate samples according to treatment, since PC1 separate infected and water-injected fruits (mostly positive scores) from control fruits (negative scores). According to PCA loading plot (Fig.

5b), PC1 was positively correlated with TP, PAL and intensity of lipid peroxidation (LP), while others biochemical assays had shown negative correlation. LP had the most important positive correlation whereas TRC, NBT, SOD and APX had shown the strongest negative correlation with PC1. The second principal component (PC2) accounted for 23.31 % of the total variance. It is likely that this component reflects the effect of genotype on the analysed parameters, considering that it separated Una and Kurtovska kapia (mostly positive scores) from Amfora fruits (mostly negative scores). PC2 showed a positive correlation with most of the assayed parameters, except for CAT, GPX, APX. As could be seen in Fig. 5b, ascorbic acid had the highest positive, while CAT had the highest negative influence on PC2.

#### CONCLUSION

Considering that the intensity of lipid peroxidation, as biological marker of oxidative stress, was increased in the wounded and sterile water-injected fruits as well as in infected fruits with *A. alternata*, it can be concluded that these factors disturb redox balance in peppers fruits in all examined genotypes. The pericarp thickness had the lowest value for Kurtovska kapia and was statistically different from Amfora and Una. Although the largest lesion developed on the surface of Una, its size was not significantly different from the lesion on Kurtovska kapia. Therefore, this may indicate that the pericarp thickness is not related to the defence network of pepper fruits as structural barrier. In present work, certain differences among genotypes exposed to wounding and pathogen stress were observed. In inoculated Amfora and Una fruits, TP and AA increase compare to control fruits. Given that, it can be concluded that mentioned phytochemicals have a contribution to the defence mechanism of Amfora and Una fruits against pathogen. In Kurtovska kapia level of vitamin C decreased after inoculation, while TP content was not changed either by wounding or inoculation. Except for PAL activity in Amfora and Kurtovska kapia and APX activity in wounded Kurtovska kapia fruits, all measured enzyme activity showed no changes or decrease by wounding and/or pathogen infection.

#### SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/12128>, or from the corresponding author on request.

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ИЗВОД  
АНТИОКСИДАТИВНИ ОДГОВОР ПЛОДОВА СЛАТКЕ ПАПРИКЕ НА ИНФЕКЦИЈУ  
ГЉИВОМ *Alternaria alternata*

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Паприка (*Capsicum annuum*) је важан извор многих биоактивних једињења, са заштитном улогом у биљкама од биотичког и абиотског стреса, и има благотворно дејство на здравље људи. Ово поврће је подложно многим инфекцијама, укључујући трулеж плодова након бербе проузроковано гљивом *Alternaria alternata*. У циљу бољег разумевања одбрамбеног система плодова, у раду је мерена концентрација фенола и аскорбинске киселине, антиоксидативна ензимска и неензимска активност у плодовима слатке паприке типа капија (сортe: Амфора, Уна и Куртовска капија) заражене гљивом *A. alternata*. Плодови Амфоре су имали највећу толерантност према инфекцији. Садржај укупних фенола и витамина С повећао се у плодовима Амфоре и Уне након механичког оштећења и инокулације, док су плодови Куртовске капије у та два случаја смањили количину витамина С. У зависности од механизма реакције антиоксидативни тестови нису показали промене ни смањење антиоксидативног капацитета третираних плодова. Осим активности фенилаланин-амонијум лиаза у Амфори и Куртовској капији и активности аскорбат-пероксидазе у механички повређеним плодовима Куртовске капије, све мерене активности ензима нису показале промене услед рањавања и/или инфекције гљивом *A. alternata*. Према резултатима интензитета липидне пероксидације као биолошког маркера оксидативног стреса, може се закључити да механичке повреде и инфекција нарушавају редокс равнотежу код свих испитиваних генотипова. Утврђено је да испитивани генотипови показују извесну разлику у антиоксидативној одбрани од механичког оштећења и патогена.

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