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INFLUENCE OF LATE BLIGHT (PHYTOPHTHORA INFESTANS) ATTACK ON NUTRITIONAL QUALITY OF TOMATO

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ABSTRACT

The effect of natural infection with Late Blight (Phytophthora infestrans) on chemical fruit quality on six tomato (Lycopersicon esculentum) varieties (Menhir F1, Lady Rosa F1, Kilates F1, Anthalia F1, Prekos F1, Vanessa F1) was investigated in a field trial. Plants were exposed to stress produced by natural infection with P.infestans and biochemical quality parameters were analyzed as well as antioxidant activities for both treated and untreated variants. P.infestans attack on tomato was associated with lower total soluble solids and sugar content and an increased acidity. There was no significant correlation between lycopen content and pathogen attack. Antioxidant activity reflected by PPH radical scavenging activity and ABTS radical cation scavenging activity increased in case of pathogen`s attack.

INTRODUCTION

Plants are subject of various biotic and abiotic stresses due to unfavorable environmental conditions that affect their growth, metabolism and yield (Dumas et al., 2003; Atkinson et al., 2011). *Phytophthora infestans* is one of the most devastating tomato pathogen which are difficult to control being able to devastate all crop within 7 to 10 days (Fry, 2008). Economic losses may be in the form of reduced yield, lower quality of the fruit, diminished storability and increased cost associated with fungicide application (Nowicki et al. 2012).Tomato fruits infection may range from 41% to 100% on untreated plots and from 12% to 65% in plots protected with systemic fungicides (Fontem et al. 1996). Therefore control of Late Blight (LB) is indispensable and the LB management includes cultural practices, fungicide sprays and use of resistant cultivars. Previous studies showed that considerable efforts have been made to identify genetic source of LB resistance and transfer resistance to modern breeding lines and cultivars (Brouwer and St Clair, 2004; Fooland et al.2006). All these efforts emphasise the importance of keeping healthy tomato crops well known for their nutritional qualities. The levels of nutritional components in tomatoes depend on cultivar, ripening stage, growth stage, growth conditions and environmental influence.

Pathogens stress elicits a complex cellular and molecular response system implemented by the plant in order to prevent damage and ensure survival, but often at the detriment of growth and yield (Herms and Mattson, 1992). Plants can respond and adapt to diferent type of stress by altering their cellular metabolism and invoking various defense mechanism (Atkinson et. al.,2011).One of the earliest responses of plants to pathogen stress is the accumulation of reactive oxygen species which can be extremely reactive, especially singlet oxygen and the hydroxyl radical and, unlike atmospheric oxygen, they can oxidize multiple cellular components like proteins, lipids and nucleic acids.This enhanced reactive oxygen species production is scavenging by a versatile antioxidant system that modulates intracellular concentration and sets the redox-status of the cell. The major scavenging mechanisms include enzymatic antioxidant systems and non-enzymatic

antioxidants (Suzuki et al, 2014). Phenolic compounds are of interest nutritionally, as when present in the diet they confer health benefits related to their antioxidant activity.Plants produce phenolic compounds as a defensive mechanism in response to attack by pests or pathogens such as insects, fungi, or nematodes (EnglishLoeb et al.,1997;Treutter,2006) and in response to abiotic stresses.However, little work has been done for examination of pathogens attack on the nutritional quality of tomato. The main goal of the current study was to determine the effect of *P.infestans* stress on the levels of quality chemical quality parameters in tomato fruits. For a better evaluation it was made a comparative assessment between chemical quality parameters recorded on treated tomato fruits which recorded an attack degree below economical threshold and those recorded on untreated tomato fruits.

MATERIAL AND METHODS

Field experiment was carried out on Experimental Research Station Banu Maracine (luvosoil, pH 6,06-6,47) during 2012 year in order to evaluate de influence of *Phythophtora infestans* attack, under natural conditions, on the nutritional quality of six tomato cultivars. The experiment was a split plot design with two factors x three replications. Each plot size was 10 m^2. The trail factors were: Factor A: tomato hybrid (V1-Menhir F1, V2-Lady Rosa F1, V3-Kilates F1, V4-Anthalia F1, V5-Prekos F1, V6-Vanessa F1) and Factor B: fungicide treatment. Fungicide treatments were made according with the following scheme:the 1^{st} treatment – Dithane M 45 (mancozeb 80%) -0,2%; the 2^{nd} treatment – Shavit F72WP (folpet 70%+triadimenol 25) -0,2%; the 3^{th} treatment – Ridomil Gold Plus 42,5WP (cupru 40%+mefenoxam 2,5%) -0,3%; the 4^{th} treatment – Bouille bordelaise -0,75%.

Control plants were not spayed with fungicide.First treatment was done on late April and next ones were applied every 14 days.In all trial the modified rating scale (Horsfall and Barratt, 1945) was used to rate individual plants for disease severity. The attack degree (AD %) of *P. infestans* was calculated for each variant according with formula AD% = (Severity% x Intensity %)/100 (Savescu et al., 1969). For the analysis of nutritional compounds, three plants were sampled per treatment group. Fresh healthy and attacked tomato fruits were assessed for following chemical components:total soluble solids, reducing sugar, acidity (% as citric acid), ascorbic acid, lycopene content. Antioxidant activity was evaluated following free fenolic content, DPPH radical scaveging activities and ABTS radical cation scaveging activities.*The soluble solids content (SSC)* ***%*** was determined using a digital refractometer.*The titratable acid content(acidity)* was determined by titration with 0.1N sodium hydroxide (NaOH) and expressed as % citric acid. *Reducing sugars (%)* were extracted in distilled water (1:50 w/V) and assayed colorimetric with 3,5 dinitrosalicylic acid according to Miller (1959).*Ascorbic acid* was extracted in 2% hydrochloric acid (HCl) (1:50 w/V) and determined by iodometric titration.*Lycopene* extraction and determination were conducted as described by Fish et al. (2002) *Determination of total phenolic content (TPC):* Extracts for the determination of phenols and antioxidant activity were prepared into 80% aqueous methanol (1:10 w/v) at 24°C for 16 h. Phenolic compounds were determined colorimetric by using the Folin-Ciocalteu method (as described by Dannehl et al.,2011) based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. *DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay:* The capacity of tomato extracts to reduce the radical 2,2-diphenyl-1-picrylhydrazyl was assessed using the method of Babbar et al, 2014 with some modification. A 0.075 mM (final concentration) DPPH solution in ethanol was mixed with sample extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 20 min at 2 min intervals. The absorbance of the remaining DPPH radicals was measured at 519 nm. The normal color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. A blank reagent was used to study stability of $DPPH^{\cdot}$ over the test

time. The scavenging activity of extracts was evaluated as a percentage of DPPH discoloration using the formula: % scavenging = $[A_0-(A_1–A_S)]/ A_0]100$, where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract only. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram. (µmol TE/g fw).*ABTS radical cation scavenging activity:*The ABTS radical cation scavenging activity of the methanolic extract was measured using the method ofPellegrini et al.(2007) with some modifications. ABTS was dissolved in water to a 7 mmol/l concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/l potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. 0.1 ml of sample extract was mixed with 2.9 ml of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of fresh material. (µmol TE/g fw). All determinations were performed in triplicate, and all results were calculated as mean.

RESULTS AND DISCUSSION

The environmental conditions of 2012 year were highly conductive to Late Blight (LB) development. During April – September interval the air temperature was higher with 2,1^{o}C than multiannual average. Heavy rain falls in April (79,3 mm) and Mai (136,7 mm) generated favorable conditions for *Phytophthorainfestans* infection and rapid development. The highest attack degree on fruits was observed on the hybrids Prekos F1 and Vanessa F1 (Table 1).Tomato hybrid Kilates F1 (V3) didnt reach the maturity due to the attack of Cucumber Mosaic Virus. The variants fungicide treated didn't emphasized *P.infestans* attack sympthoms on fruits.

Sugar and organic acids are the main metabolites in tomato fruits and constitute over 60% of the dry matter. The higer level of soluble solids, reducing sugar and acidity can positively influence the flavor quality of tomatoes (Auerswald et al, 1999). Total soluble solids (TSS) were significantly affected by the pathogen attack emphasizing lower levels for untreated tomato fruits. TSS values ranged between 4,1% and 5,5% for treated variants and between 3% and 5% for untreated variants (Table 1).Sugar reducing concentration was also heightened by *P.infestans* attack ranging between 3,31% (V1-Menhir F1) and 4,2% (V2-Lady Rosa F1) while on affected variants reducing sugar content decreased. Among all studies tomato hybrids V2- Lady Rosa recorded the highest levels of total soluble solids (5,5%) and reducing sugar (4,2%). These findings are similar with previous results obtained by Bastias et al. (2011) and Pinela et al. (2012). Generally, it was observed a depression of reducing sugar content to all attacked tomato fruits.

Acidity in tomato is attributed mainly to citric acid and malic acid (over 90% of the organic acid). It was observed that acidity is higher on variants attacked by *P.infestans* and no treated. This can be explained by sugar bioconversion into organic acids as a result of pathogen attack. These results confirm previous research of Toor et al. (2006), Dannehl et al. (2014). Ascorbic acid values ranged between 10 mg/100g (V2-Lady Rosa F1) and 15,01mg/100g (V5-Prekos F1) for treated tomato fruits and between 10,44 mg/100g (aV2-Lady Rosa) and 19,5 mg/100 g (aV6-Vanessa F1) for tomato fruits exposed to *P.infestans*attack. The data reported in the literature were higher than current study data (Ilahy et al. 2011), but similar to those reported by other results (Toor et al, 2006).

Table 1

Biochemical indices determined for treated variants (Vx)* and for tomato variants affected by *P.infestans* (aVx)**

Variant	**Attack Degree on fruits (AD %)**	**Total soluble solids (%)**	**Reducing sugar (%)**	**Ascorbic acid (mg/100g)**	**Acidity (% as citric acid)**	**Lycopene (mg/kg)**
V1	0	4.1	3.31	12.18	0.29	73.64
aV1	7,20	4	3	12.66	0. 57	53.74
V2	0	5.5	4.2	10	0.28	65.05
aV2	9,31	5	2.45	10.56	0. 31	72.8
V4	0	5.1	3.86	12.66	0.26	49.95
aV4	4,67	4.2	3.52	10.44	0.55	42.42
V5	0	4.2	3.49	15.01	0.25	60.06
aV5	11,30	5	3.4	14.61	0. 35	79.38
V6	0	5	3.87	11.88	0.38	84.92
aV6	15,42	3	2.75	19.5	0.56	65.876

*fungicide treated variants (healthy tomato fruits)

**no treated variants (variants affected by *P.infestans – diseased tomato fruits*)

It was observed that studied tomato hybrids had high lycopen content leading to the conclusion that tomato fruits present good healthy beneffits. Lycopene accounts for 80 - 90% of total carotenoids in tomato and when consumed is associated with a reduction in the risk of prostate and other cancers, as well as protection against cardiovascular disease (Rao and Agarwal, 2000). The concentration of lycopen in tomato samples varied between 49,95 mg/kg (V4-Anthalia F1) and 84,92 mg/kg (V6-Vanessa F1) for treated variants, while for diseases variants lycopen concentration ranged between 42,42 mg/kg (aV4-Anthalia F1) and 72,8 mg/kg (aV2-Lady Rosa F1). These results are similar with previously findings (Ilahy et al. 2011, Dannehl et al, 2014). It wasnt noticed a significant correlation between lycopen content and pathogen attack.

Total phenolic content showed higher levels for diseased variants comparatively with those treated with fungicides due to antioxidant character of these components (Fig.1).Total phenolic content ranged between 287.23 µg GAE/g fw (V5-Prekos F1) and 353.98 µg GAE/g fw (V1-Menhir F1) for treated variants and between 310.96 µg GAE/g fw (aV6-Vanessa F1) and 568.36 µg GAE/g fw (aV5-Prekos F1) for diseased variants.Similar results were reported by other studies (Ilahy et.al. 2011;García-Valverde et al. 2013; Pinela et al. 2012; Barros et al. 2012).

DPPH radical scavenging activity showed high values for all experimented variants (Figure 1). The values of DPPH radical scavenging activity ranged between 928.2 µmol TE/gfw (V6-Vanessa F1) and 1410.8 µmol TE/gfw (V1-Menhir F1) for treated variants and between 1015.4 µmol TE/gfw (aV6-Vanessa F1) and1805.6 µmol TE/gfw (aV1-Menhir f1) for diseased variants.

Tomato samples were also measured and compared for their free radical scavenging activities against ABTS radical cation. All studied variants showed significant ABTS radical cation scavenging activity. The values of ABTS radical cation scavenging activity rangedbetween 1527 µmol TE/gfw (V5) and 1949 µmol TE/gfw (V2) for treated variants and between 1783 µmol TE/gfw (aV6) and 2384 µmol TE/gfw (aV1) for variants affected by pathogen.

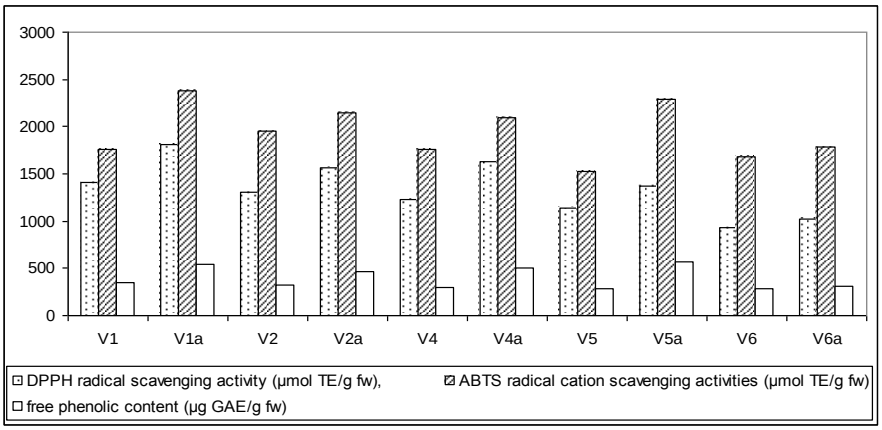

***Figure 1. Free phenolic content (µg* GAE/g fw), DPPH radical scavenging activities (µmol TE/g fw) and ABTS radical cation scavenging activities (µmol TE/g fw)**

Positive correlations have been observed when DPPH radical scavenging activity and ABTS radical cation scavenging activity were compared with free phenolic compounds content, thus indicating that these compounds are responsible for the antioxidant activity.

CONCLUSIONS

Each stress biotic and abiotic factor requires an unique mechanism of response, tailored to the specific needs of the plant.Metabolic and signaling response of plants involve frequently antioxidant mechanism and few biochemical indices alteration.The attack of *P.infestans* to tomato fruits affects directely nutritional quality components. Total solube solids and reducing sugar were significantly affected by pathogen`s attack. It was observed also in increased acidity to diseased tomato fruits. Antioxidant activity reflected by PPH radical scavenging activity and ABTS radical cation scavenging activity increased in case of pathogen`s attack as a result of activation of enzymatic and non-enzymatic antioxidant system in order to maintain cellular redox status. Increased phenolic content levels and positive correlation between phenolic content and DPPH radical scavenging activity and ABTS radical cation scavenging activity is explained by antioxidant character of these components.

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