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Processing tomato response to arbuscular mycorrhizal fungi application under conventional production practice

Odgovor industrijske rajčice na primjenu arbuskularnih mikoriznih gljiva u uvjetima konvencionalne proizvodnje

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ABSTRACT

The potential effect of pre-inoculation of processing tomato (*Solanum lycopersicum* L.) plants with arbuscular mycorrhizal fungi (AMF) on yield and fruit properties was investigated in conventional production. Tomato seeds were sown in seedling trays filled with a substrate non-inoculated (AMF-) or inoculated (AMF+) by selected mycorrhizal strains. Seedlings were transplanted in *Terra rossa* soil and grown in conditions with mineral fertilization and irrigation. Tomato fruits were sampled at harvest. Tomato plants under both treatments had high levels of mycorrhizal colonization, due to applied inoculum (AMF+) or indigenous (AMF-) inoculum present in the soil. Applied AMF+ treatment increased P and decreased K content in tomato fruits. Content of trace elements such as As and V significantly increased, while Pb content significantly decreased in the fruits of AMF+ when compared to the AMF- treatment. No significant effect of AMF+ treatment was observed on yield, fruit quality (soluble solids, pH, total acidity, fruit firmness), lycopene and antioxidant activity of tomato fruits. The overall results suggest that processing tomato is highly susceptible to the indigenous AMF, while seedlings inoculation with selected AMF improves only total phenolic and P fruit content. This means that AMF have a potential application in commercial processing tomato production, however a targeted adaptation of management decissions is required for more extensive results.

Keywords: antioxidant activity, fruit quality, *Funneliformis mosseae*, lycopene, mineral acquisition, mycorrhizal colonization, Rhizophagus irregularis, tomato seedlings, total phenolic content, trace elements

SAŽETAK

Potencijalni utjecaj inokulacije presadnica industrijske rajčice (*Solanum lycopersicum* L.) selekcioniranim arbuskularnim mikoriznim gljivama (AMF), na prinos i svojstva ploda, istražen je u konvencionalnoj proizvodnji. Sjeme rajčice posijano je u polistirenske kontejnere s neinokuliranim (AMF-) ili mikorizama inokuliranim (AMF+) supstratom. Presadnice su posađene

u crvenicu, u proizvodnim uvjetima s mineralnom gnojidbom i navodnjavanjem. Uzorkovanje plodova industrijske rajčice provedeno je u berbi. Kod oba tretmana utvrđen je visoki postotak kolonizacije selekcioniranim mikorizama (AMF+) i nativnim vrstama (AMF-) prisutnim u tlu. Plodovi rajčice s AMF+ biljaka imali su veći sadržaj P i manji sadržaj K u odnosu na AMF- tretman. Sadržaj As i V bio je signifikantno veći, dok je sadržaj Pb bio signifikantno manji u AMF+ u usporedbi s AMF- tretmanom. Signifikantan učinak tretmana AMF+ na prinos, kvalitetu ploda (suha tvar, pH, ukupna kiselost, čvrstoća ploda), likopen i antioksidacijsku aktivnost je izostao. Temeljem dobivenih rezultata možemo zaključiti da je industrijska rajčica podložna nativnim AMF, dok je ciljana inokulacija presadnica AMF značajno povećala ukupni sadržaj fenola i P. Dobiveni rezultati ukazuju na potencijalnu primjenu AMF u komercijalnoj proizvodnji industrijske rajčice, međutim prethodna prilagodba uzgojnih uvjeta je neophodna za postizanje značajnijih rezultata.

Ključne riječi: antioksidativna aktivnost, elementi u tragovima, *Funneliformis mosseae*, kolonizacija mikorizama, kvaliteta ploda, likopen, presadnice rajčice, *Rhizophagus irregularis*, sadržaj ukupnih fenola, usvajanje minerala

INTRODUCTION

Processing tomato (*Solanum lycopersicum* L.) production, in regions where limited production area is available, is often associated with insufficient crop rotation, intensive pesticide application and mineral fertilization. In Istria County (Croatia), growing of processing tomato has long tradition and the majority of it is in areas where *Terra rossa* soil prevails. Natural characteristics of the *Terra rossa* soil are associated with low organic matter content and lower content of plant available phosphorous (P) (Bašić, 2013). Moreover, the bioavailability of P in *Terra rossa* soil is very limited as a result of its retained diffusion and pronounced fixation with elements like iron (Fe) (Gupta et al., 2014). In this type of soil, a frequent demand for improvement of P content and soil organic matter content is observed, which is fundamental in processing tomato production (Hartz et al., 1999; Elia et al., 2006). A high demand for P addition in conventional production leads to excessive fertilizer application and contributes to significant soil deterioration in long term (Ju et al., 2007; Ren et al., 2010). In addition, frequent application of mineral fertilizers is associated with soil contamination with minor amounts of impurities attributed to trace elements, such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) (Gupta et al., 2014). Application of arbuscular mycorrhizal fungi (AMF) could foster a more sustainable processing tomato production under conditions of intensive agriculture.

Arbuscular mycorrhizal fungi constitute a group of obligate biotrophs that form a symbiosis between plants and fungi (Fungi: Glomeromycota) (Berruti et al., 2016). They provide a range of benefits to host plants, such as well-known enhancement of water and nutrients supply (Gosling et al., 2006; Jansa et al., 2008; Parniske, 2008; Baum et al., 2015). An efficient interaction between the mycorrhizal species and processing tomato plants improves the absorption of essential but commonly deficient macro- and micronutrients (Conversa et al., 2007; Conversa et al., 2013; Bowles et al., 2016). There is a limited data available on the benefits of mycorrhizal application on processing tomato fruit nutrient content (Antunes et al., 2012), however, numerous investigations were conducted on mycorrhizal application in other crops (Ban et al., 2011; Ortas, 2012; Baum et al., 2015; Cavagnaro et al., 2015; Berruti et al., 2016). More precisely, it is well known that AMF have a positive impact on the intake of macro- [calcium (Ca), nitrogen (N), magnesium (Mg), P, potassium (K)] (Clark and Zeto, 2000; Giri and Mukerji, 2004; Hajiboland et al., 2010) and micronutrients [boron (B), copper (Cu), Fe, manganese (Mn), zinc (Zn)] (Clark and Zeto, 2000; Miransari, 2010; Ceci et al., 2012). Furthermore, not only AMF promote the intake of plant-required nutrients but also alleviate the negative effects of different trace elements that could be a limitation for plant production [aluminum (Al), arsenic (As), Cd, cobalt (Co), Cr, sodium (Na), Ni, Pb, antimony (Sb), selenium (Se), strontium (Sr), vanadium (V)] in field production of numerous crops (Roger and Williams, 1986; Clark and Zeto, 2000; Suzuki et al., 2001; Davies et al., 2002; Jamal et al., 2002; Giri and Mukerji, 2004; Liu et al., 2005; Hilderbrandt et al., 2007; Miransari, 2010; Ceci et al., 2012; Kumar et al., 2015; Wei et al., 2016).

Finally, in terms of processing tomato production, artificial inoculation with known strains of AMF can significantly contribute to the improvement of yield and qualitative parameters, such as the nutritional value or flavor (Hartz et al, 1999; Conversa et al., 2007; Conversa et al., 2013).

The aim of the present study was to investigate the effects of artificial inoculation of processing tomato seedlings, with mycorrhizal species *Rhizophagus irregularis* (syn. *Glomus intraradices*) and *Funneliformis mosseae* (syn. *Glomus mossease*), on yield and quality parameters, nutrient and trace element fruit content under common commercial growing technology.

MATERIALS AND METHODS

Plant material

The seedlings of commercial tomato hybrid Perfect Peel (Seminis, Monsanto Agricoltura Italia S.p.a., Milano, Italy) were prepared in bulk in Grunt nursery, located in Varaždin, Croatia.

The Aegis microgranules, as a granulated inoculum of *R. irregularis* and *F. mosseae* (Atens, Tarragona, Spain), were thoroughly mixed with the substrate Klasmann Potgrond P® (Klasmann-Deilmann GmbH, Geeste, Germany) at 20 g/L substrate. At the same time a non-mycorrhizal substrate was prepared.

Pelleted tomato seeds were sown on 14th March 2017 in seedling trays with 209 cells (21 mL volume per cell), one seed per cell. The following treatments were applied: i) seedlings grown in mycorrhizae inoculated substrate (AMF+), ii) seedlings grown in substrate without mycorrhizae inoculation (AMF-). Transplants were grown in a heated greenhouse under natural light conditions. Seedling trays were irrigated as required by a sprinkler system.

Soil analysis

Soil chemical properties were determined according to previously defined methods (Pasković et al., 2019). Samples for chemical analysis were obtained from 0 – 30 cm soil depth. Analysis was conducted for soil pH (HRN ISO, 2005), total nitrogen (HRN ISO, 2004), organic matter (ISO, 1998), available phosphorus (P) and potassium (K) as described by the Egner-Riehm-Domingo method (Egner et al., 1960).

The soil selected for the field experiment had the following properties: pH in H_2O suspension 6.7, pH in KCl suspension 5.4, total N content 0.1%, available P 13.1 mg/100 g and K 32.1 mg/100 g, and organic matter 2.3%.

Field experiment

Produced transplants were transported to the field of a commercial grower with extensive experience in processing tomato production. The growing site was located in Umag (45°25'N, 13°32'E; Istria County, Croatia). The field experiment was carried out during the growing season 2017 in the red soil *Terra rossa* (Rhodic Cambisol, IUSS Working Group WRB, 2015) under conventional production system. Ameliorative fertilization was applied two months before seedlings transplantation at following rates: P at 160 kg/ha, K at 240 kg/ha, and N in the form of Urea at 69 kg/ha.

Transplantation of mycorrhizal and non-mycorrhizal tomato seedlings was performed on 1st of May 2017. The experiment was arranged in a completely random design with four replications. Additional foliar N and K fertilization was applied during the growing season as follows: single application of Tropicote (Yara International ASA, Oslo, Norway) N fertilizer in dose of 100 kg/ha a month after seedlings transplantation, dual application of HakaPhos Violeta NPK 13:40:13 (Compo Expert, Spain) in dose of 250 g 100/L, and triple application of Idron NPK 10:5:40 (K Adriatica Group, Italy) in dose of 250 g 100/L.

Fruit firmness

Tomato fruit firmness was measured on 20 fruits per repetition, 80 fruits per treatment using the PCE - PTR-200 penetrometer (PCE Instruments, Palm Beach, USA) equipped with a 7.9 mm diameter plunger. Obtained results were expressed in kg/cm^2.

Fruit sampling

Tomato fruits were harvested at commercial maturity and samples of 20 mature tomato fruits per replicate were collected randomly at 10th August 2017, in approximate with the mechanical harvest. There were 80 mature tomato fruits in total per treatment used for further fruit analysis. Half of the sampled tomato fruits was prepared for mineral analysis, while the other half was separately prepared for the overall analysis of quality parameters, lycopene, total phenolic content, and antioxidant activity.

Fruit sample preparation

Mineral analysis

Fresh fruits, collected for further mineral analysis, were rinsed with tap water, cut and dried at 60 °C using the Memmert Universal Oven UF160 (Memmert GmbH + Co. KG, Schwabach, Germany) until reaching the constant mass. Afterwards, samples were finely grounded in a mill (Conrad Electronic, Hirschau, Germany) until a homogenized powder was obtained.

Quality parameters, lycopene analysis, total phenolic content, and antioxidant activity

Samples of fresh tomato fruits were rinsed with tap water, cut into fourths and frozen at −20 °C (GT 4232, Liebherr, Bulle, Switzerland) until further analyses of quality parameters, lycopene, total phenolic content and antioxidant activity. Tomato samples were defrosted at room temperature, cut in smaller cubes and finely grounded to a puree with an electric hand blender (BOSCH, model MSM7400, Stuttgart, Germany). The homogenized tomato puree samples were used for determination of pH, titrable acidity, soluble solids, and dry matter. Hydrophilic and lipophilic extracts of tomato puree samples were prepared according to Fanasca et al. (2006) and subjected to further analysis for the total phenolic content and antioxidant activity.

Chemicals

Deionised water was obtained with Elix 3 purification system (Millipore, Merck KGaA, Darmstadt, Germany). Ethanol, 96% p.a. was a product of Alkaloid Skopje (Skopje, Republic of North Macedonia). Folin-Ciocalteu reagent and sodium carbonate were obtained from Kemika (Zagreb, Croatia). Methanol and acetone were of chromatography grade both products of Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Chromatography grade hexane was a product of BDH prolabo (VWR International Ltd, Lutterworth, UK). Trypan blue, 2,2-Diphenyl-1-picrylhydrazyl (DPPH•), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 3,5-Di-tert-4-butylhydroxytoluene (BHT), and gallic acid were all products of Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Quality parameters

All measurements were made in duplicates. The principles from AOAC International (1980 a – c) methods of analysis were followed, with minor modifications as specified here. For the determination of the dry matter content (DM), 5 ± 0.1 g of homogenized tomato sample was dried in a convention oven at 105 °C until constant weight. pH of homogenized tomato purees was measured at room temperature with a pH meter (MP220 Basic pH/mV/°C Meter, Mettler-Toledo GmbH, Gießen, Germany), after calibration with certified pH buffers. The soluble solids content was measured at room temperature with a digital HR200 Portable refractometer (Artisan, APT Instruments, Minneapolis USA). Readings were expressed as Brix degrees. For determination of total acidity, 10 g of tomato puree was accurately weighed into 250 mL bakers, and 200 mL of deionised water was added. The resulting mixture was titrated with 0.1 M NaOH using a digital Burette (Solarus 20 mL, Hirschmann Laborgeräte GmbH & Co., Eberstadt, Germany) to a pH value of 8.0 measured with a pH meter. Total acidity was calculated as a percentage of citric acid on a fresh weigh basis (% CA/FW).

Mineral analysis

Content of P was evaluated by UV-VIS spectrophotometer (Carry UV/Vis 50, Varian, Palo Alto, USA), following the method proposed by Miller (Miller, 1998). Moreover, according to the same author K, Ca, Mn

and Na content were analyzed by PerkinElmer AAS800 flame atomic absorption spectrometer (PerkinElmer Instruments, Waltham, MA, USA). Analyses of other macro-, micronutrients and trace elements (Mg, Fe, Cu, Zn, B, Cr, As, Cd, Ni, Pb, Be, Sb, Co, Se, V, Ba, Ag, Mo, Li, Sr) were performed using an inductively coupled plasma mass spectrometer (ICP-MS) NexION 300x (PerkinElmer Instruments, Waltham, USA).

Lycopene analysis

The amount of lycopene in tomato puree samples was determined by spectrophotometry as proposed by Fish et al. (2002). Tomato purees were diluted with deionised water, 1:1 (v/v) in order to get spectrophotometer readings in a reliable range (0.1 – 0.8 AU), where the dilution was considered for calculation of lycopene content. Homogenized tomato puree samples were weighed into a flask to which was immediately added water for dilution, and 20 mL of a solution composed of hexane, ethanol, and acetone in volume proportion equal to 1:2:1. The absorbance of the hexane layer (top) was measured at 503 nm using a Carry UV/Vis 50 spectrophotometer (Varian Inc., Harbour City, USA), and pure hexane was used as reference. The content of lycopene (mg/kg of tomato on fresh weigh basis) was calculated using the molar absorptivity of lycopene in hexane.

Total phenolic content

Total phenolic content (TP) in HP and LP extract of tomato samples was estimated based on the coloration reaction of phenols with the Folin–Ciocalteu reagent and sodium carbonate, and following spectrophotometric reading of light absorbance at 725 nm (Carry UV/Vis 50, Varian Inc., Harbour City, USA). Gallic acid was used as reference.

Antioxidant activity

The determination of Free radical scavenging capacity was performed using the stable radical DPPH•, relying on principles of methods described in Brand-Williams et al. (1995) and Sánchez-Moreno et al. (1998) that is the fact that scavenging of free radicals by samples or standards causes a reduction in DPPH• absorbance. Minor modifications and adaptation of the method is described in the following text.

Trolox was used as reference. Stock solution was of content of 200 µM (0.05 g/L) and standard solutions were prepared in the range from 10 to 100 µmol. Results were expressed as mmol Trolox equivalents (mmol TE 100 g/FW).

A sufficient amount of the radical DPPH• solution in methanol was prepared, in content of 0.025 g/L. One hour after addition of samples/standard solution/blank (0.3 mL for HP; 1.0 for LP) to DPPH· solution (4.7 mL for HP; 4 for LP), the percentage of remaining DPPH• was determined by reading absorbance at 515 nm end calculated using the regression equation between the Trolox content and the percentage of DPPH inhibition obtained from the calibration curve.

Determination of root mycorrhizal colonization

Root samples were taken randomly on 10th May, 2017 and at the end of the experiment 10th August 2017. Tomato roots were washed with tap water, cleaned with 10% KOH w/v, acidified with 1% HCl w/v and colored with trypan blue. Prepared samples were stored in glycerol until analysis, when they were observed under a light microscope (Philips and Hayman, 1970). Root fragments were cut and arranged on slides and examined under light microscope (magnification 200×; Axio Plus; Carl Zeiss, Oberkochen, Germany). Total colonization of roots, arbuscular, vesicular and hyphal colonization was determined using the magnified intersections method (McGonigle et al., 1990). Between 150 and 200 root intersections per plant were analyzed.

Statistical analysis

The experiment was set up as a completely random design in four replications. Statistical analysis was performed using Statistica 13.3 (Tibco, Inc). One-way ANOVA was performed for all data and multiple comparisons of means were based on Tukey's test. Correlation coefficients were determined. Moreover,

data were analyzed by Partial Least Square (PLS) analysis.

RESULTS

Tomato fruit properties

Measured yield, quality, lycopene content

The observed tomato yield per plant was not significantly different between treatments as it resulted in 4.03 kg/plant and 3.95 kg/plant for AMF+ and AMF- plants, respectively (Table 1). Applied AMF+ treatment had no significant effect on tomato quality parameters such as fruit firmness, soluble solids, pH, and total acidity (Table 1). The tomato fruit lycopene content was not significantly modified by the AMF+ application (Table 1).

Total phenolic content and antioxidant activity

Significant differences between applied treatments were absent for total phenolic content (TP) lipophilic fraction in fresh weight, TP lipophilic and hydrophilic fractions in dry weight, antioxidant activity (AA) in lipophilic and hydrophilic fractions both in fresh and dry weight. However, an exception is observed for TP analyzed in hydrophilic fractions measured on fresh weight basis which was higher in AMF+ plants (Table 2 and 3).

Mineral content

The content of P, Ca, Mg and K in tomato fruits is presented in Figure 1. A higher content of P in tomato

Table 1. The effect of mycorrhizal application at seedling stage on yield, quality and lycopene content of processing tomato fruit at harvest

Treatment	Yield (kg/plant)	TFF (kg/cm^2)	SS (% Brix/FW)	pH	TA (% CA/FW)	Lycopene (mg/kg FW)
AMF-	4.03±0.46	4.56±0.06	4.30±0.19	4.11±0.02	0.42±0.01	77.68±3.85
AMF+	3.95±0.17	4.74±0.11	4.33±0.19	4.09±0.02	0.46±0.02	78.15±3.22

[1] Mean values followed by different letters are significantly different at P<0.05 according to Tukey's test. Data are mean ± SE (n= 4)

[2] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae; TFF, tomato fruit firmness; SS, soluble solids; TA, total acidity; FW, parameters measured on fresh weight basis

Table 2. The effect of mycorrhizal application at seedling stage on total phenol content in processing tomato fruit at harvest

Treatment	TP (H) FW mg GA/ 100g	TP (L) FW mg GA/100g	TP (H) DW mg GA/100g	TP (L) DW mg GA/100g	TP (HL) FW	TP (HL) DW
AMF-	21.41±0.16b	12.69±2.91	425.83±9.07	254.47±62.36	34.09±2.9	680.30±67.99
AMF+	23.05±0.36a	8.29±1.99	431.40±14.78	155.41±35.8	31.34±2.07	586.82±42.09

[1] Mean values followed by different letters are significantly different at P<0.05 according to Tukey's test. Data are mean ± SE (n= 4)

[2] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae; TP (H) FW, total phenolic content – hydrophilic fractions in fresh weight; TP (L) FW, total phenolic content – lipophilic fractions in fresh weight; TP (H) DW, total phenolic content – hydrophilic fractions in dry weight; TP (L) DW, total phenolic content – lipophilic fractions in dry weight; TP (HL) FW – total phenolic content – sum of hydrophilic and lipophilic fractions in fresh weight; TP (HL) DW – total phenolic content – sum of hydrophilic and lipophilic fractions in dry weight

Table 3. The effect of mycorrhizal application at seedling stage on antioxidant activity of processing tomato fruits at harvest

Treatment	AA (H) FW µmTE/100g	AA (L) FW µmTE/100g	AA (H) DW µmTE/100g	AA (L) DW µmTE/100g	AA (HL) FW µmTE/100g	AA (HL) DW µmTE/100g DW
AMF-	23.20±0.84	8.86±0.8	462.26±24.47	175.56±13.27	32.05±0.43	637.81±17.87
AMF+	24.89±1.38	10.24±1.05	464.37±19.39	190.43±16.03	35.12±1.95	654.80±21.9

[1] Mean values followed by different letters are significantly different at P<0.05 according to Tukey's test. Data are mean ± SE (n= 4)

[2] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae; AA (H) FW, antioxidant activity – hydrophilic fractions in fresh weight; AA (L) FW, antioxidant activity – lipophilic fractions in fresh weight; AA (H) DW, antioxidant activity – hydrophilic fractions in dry weight; AA (L) DW, antioxidant activity – lipophilic fractions in dry weight; AA (HL) FW, antioxidant activity – sum of hydrophilic and lipophilic fractions in fresh weight; AA (HL) DW, antioxidant activity – sum of hydrophilic and lipophilic fractions in dry weight

fruits was observed in AMF+ plants compared to AMF- ones, whereas for K content the opposite was found (Figure 1). There was no significant difference observed for Ca and Mg (Figure 1), as well as for B, Cu, Zn, Mn and Fe content in AMF+ plants when compared to AMF- ones (Figure 2).

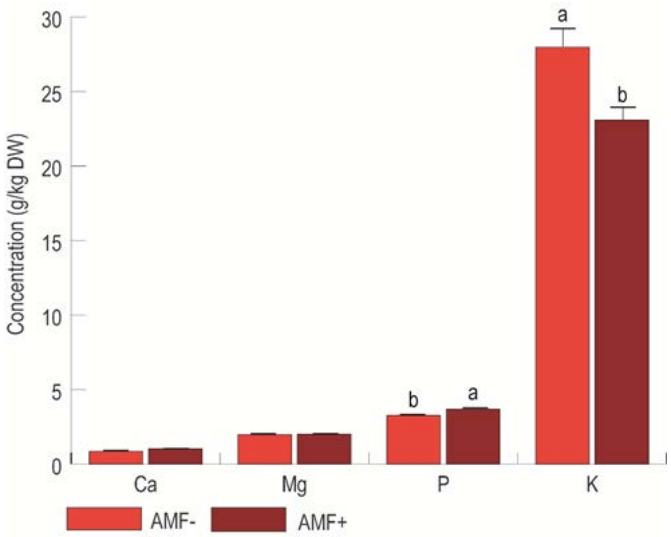
In total, 11 different trace elements were analyzed and expressed as content in dry weight (DW). The content of Pb was lower in AMF+ plants compared to non- AMF- ones, whereas the opposite was observed for As and V (Figure 3). Other analyzed trace elements showed no significant difference between the investigated treatments (Figure 3).

Mycorrhizal root colonization

The analysis of tomato roots at harvest showed generally high colonization rate (79.65%) with mycorrhizal species, however, a higher percentage of total root colonization was observed in the mycorrhizae inoculated plants (Figure 4). The arbuscular and vesicles formation was generally low in both AMF+ and AMF- (Figure 4), and it ranged from 10.86% to 13.61% and 5.83% to 6.08%, respectively. The extent of hyphal formation was abundant, but no significant difference was observed between the AMF- (63.23%) and AMF+ plants (70.83%) (Figure 4).

Overall data analysis

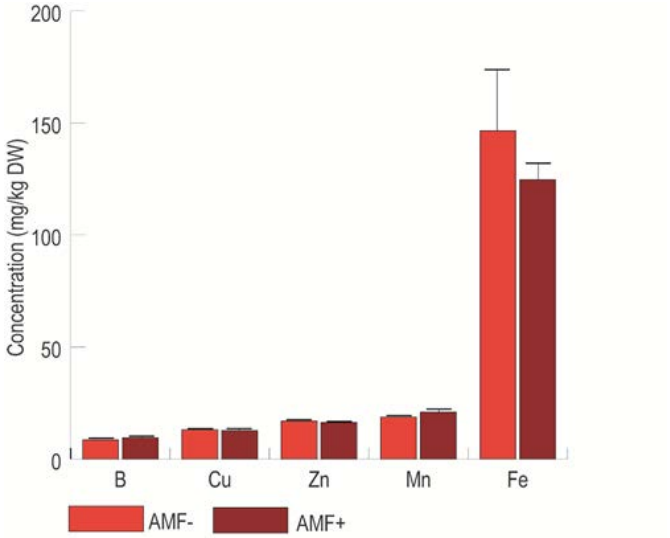
The plot obtained by processing data by PLS analysis is presented in Figure 5. The model was constructed by inspecting variable importance levels and selecting those variables with adequate predicting power. According to the obtained data all of the included parameters could be used to differentiate between the AMF- and AMF+ plants AMF+ (Figure 5). The highest predictive power in this case have V and As, followed by Pb, total root mycorrhizae colonization (TM), total phenolic content in fresh weight (TP), K and finally P (Figure 5).



[1] Mean values of the single element followed by different letters are significantly different at P<0.05 according to Tukey's test. Data are mean ± SE (n=4).

[2] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae; DW, parameters measured on dry weight basis

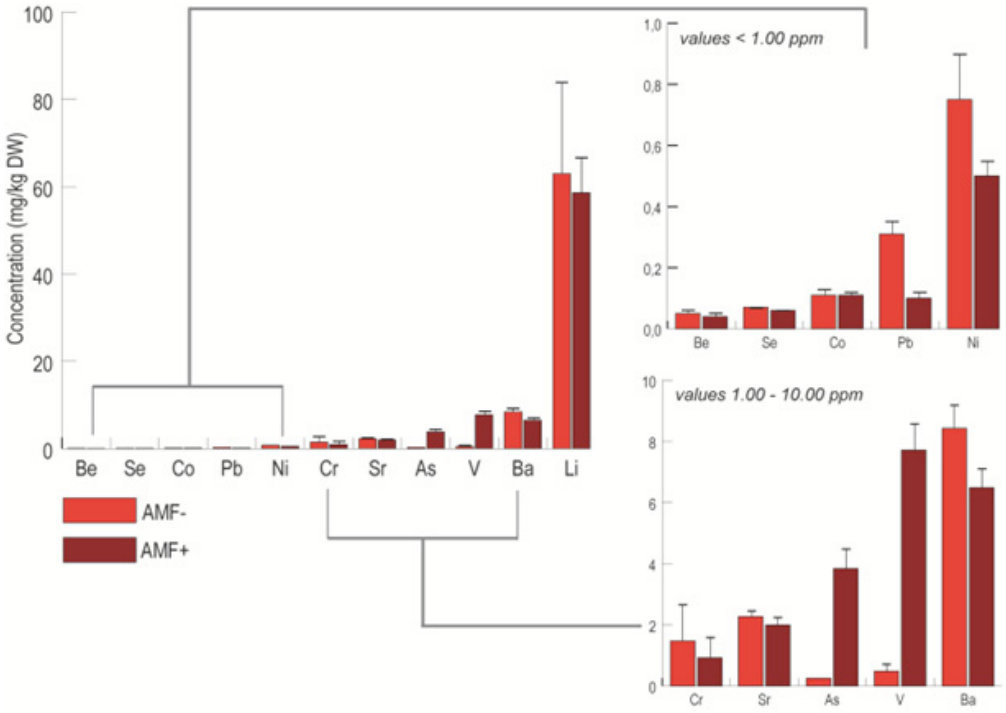
Figure 1. The effect of mycorrhizal inoculation at seedling stage on macronutrient content in processing tomato fruit at harvest



[1] Mean values of the single element followed by different letters are significantly different at P<0.05 according to Tukey's test. Data are mean ± SE (n=4).

[2] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae; DW, parameters measured on dry weight basis

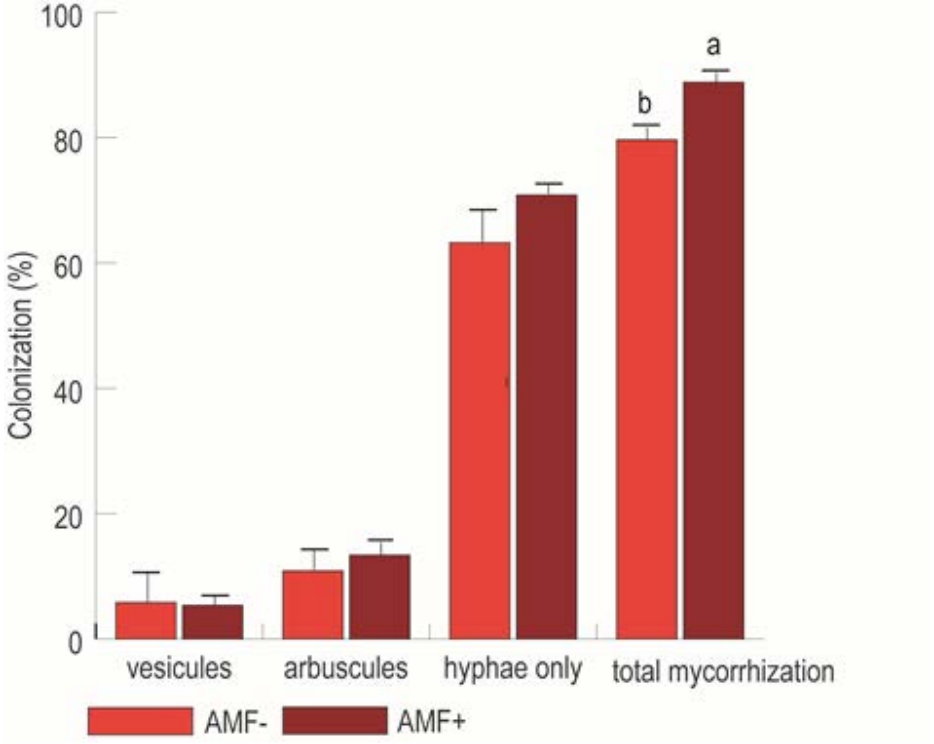
Figure 2. The effect of mycorrhizal inoculation at seedling stage on micronutrient content in processing tomato fruit at harvest



[1] Mean values of the single element followed by different letters are significantly different at P<0.05 according to Tukey's test. Data are mean ± SE (n=4).

[2] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae; DW, parameters measured on dry weight basis

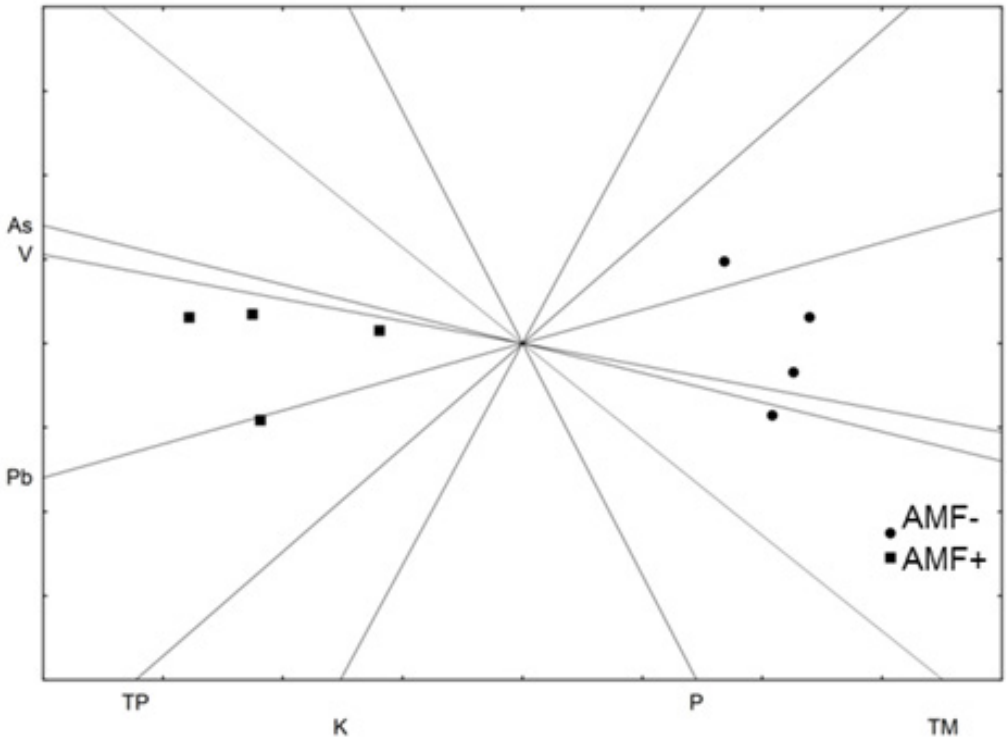
Figure 3. The effect of mycorrhizal inoculation at seedling stage on trace element content in processing tomato fruit at harvest



[1] Mean values of the colonization type followed by different letters are significantly different at P<0.05 according to Tukey's test. Data are mean ± SE (n=4).

[2] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae

Figure 4. The effect of mycorrhizal inoculation at seedling stage on mycorrhizae root colonization of processing tomato at harvest



[1] Abbreviations: AMF-, seedlings without mycorrhizal inoculation; AMF+, seedlings inoculated with mycorrhizae; TP, total phenolic content – hydrophilic fraction in fresh weight; TM – total mycorrhizal root colonization; As – arsenic; Pb – lead; V – Vanadium, K – potassium; P – phosphorus

Figure 5. PLS analysis of the results of the mineral and trace element content in processing tomato fruits, depending on the application of the mycorrhizal treatment

DISCUSSION

Our experiment was conducted during a 1-year long period, under regular farming practice, utilizing mineral fertilization, drip irrigation and fungicide treatments common in conventional production systems for processing tomato. Fertilization treatments increased both yield and quality parameters of tomato under different mineral and organic regimes when solely applied (Heeb et al., 2006; Rinaldi et al., 2007), however, these management decisions could favor or even limit the performance of mycorrhizal fungi (Suzuki et al., 2001; Miransari, 2010; Rouphael et al., 2015). Fertilization amendments applied in our experiment, correspond to the practices regularly adopted by the growers. Namely, in order to evaluate the production system as a whole, and investigate the potential benefits of AMF application on processing tomato production in such conditions, all variables present in a commercial production had to be included (Pieper and Barrett, 2009).

In our study, no yield enhancement was observed (Table 1 – 3), in contrast to a positive impact of mycorrhizal fungi on yield and fruit quality traits in fresh and processing tomato observed in other studies (Heeb et al., 2006; Ortas, 2012; Baum et al., 2015; Hart et al., 2015; Bowles et al., 2016; Helyes et al., 2017; Njeru et al., 2017; Rouphael et al., 2017; Ziane et al., 2017; Bitterlich et al., 2018; Rouphael and Colla, 2018).

Overall benefits of the applied AMF on yield, could have been absent in our study (Table 1) due to low stress level encountered in the field conditions or applied growing practices such as irrigation, fertilization regimes, etc. (Suzuki et al., 2001; Omirou et al., 2013; Hart et al., 2015). A study conducted by Bowles et al. (2016) showed that indigenous AMF increase crop yield and water use efficiency in AMF susceptible tomato genotypes grown in open field.

Among the investigated parameters of tomato fruit quality only total phenolic content, analyzed in hydrophilic fraction and measured on fresh weight basis, was increased by the mycorrhizal inoculation (Table 2). Pieper and Barrett (2009) found that differences in tomato fruit moisture content may contribute to the dilution of fruit measured parameters. However, our results were not in agreement with those reported by Pieper and Barrett (2009).

Macronutrient content of analyzed processing tomato fruits (Figure 1) were comparable with results found in other studies, with average values between Ca 0.25 - 0.38% (Olaniyi et al., 2010) and 0.12 - 0.18% (Pieper and Barrett, 2009), Mg 0.12 - 0.22% (Olaniyi et al., 2010) and 0.16 - 0.26% (Pieper and Barrett, 2009), K 0.07 - 0.17% (Olaniyi et al., 2010) and 3.22 - 4.57% (Pieper and Barrett, 2009), and P 0.38 - 0.57% (Olaniyi et al., 2010) and 0.36 - 0.44% (Pieper and Barrett, 2009). Interestingly, when we compare our results with the ones of Olaniyi et al. (2010) and Pieper and Barrett (2009), an overall difference is observed only in lower Ca content for both our treatments. In our experiment processing tomato plants had no visible Ca deficiency symptoms, thus obtained results could be genotype dependent (Njeru et al., 2017).

A significantly higher content of P (3.68 g/kg) was observed in the fruit of mycorrhizae-inoculated plants (Figure 1). Correspondingly, similar findings were reported in other studies (Al-Karaki and Hammad, 2001; Conversa et al., 2007; Conversa et al., 2013; Ortas et al., 2013; Hart et al., 2015). As others reported (Feddermann et al., 2010; Conversa et al., 2013), high total mycorrhizal colonization can increase the P uptake even when significant arbuscular formation is absent (Figure 1, Figure 4). Finally, a strong correlation between P and K uptake is usually observed (Al-Karaki and Hammad, 2001; Garcia and Zimmermann, 2014), while an opposite result is obtained in our study for their concentrations (Figure 1). However, when the total amount of P and K per plant is analyzed, there is no significant difference between the elements (data not shown). Moreover, our results show a very strong positive correlation between Pb and K ($r = 0.82$, $P<0.05$), strong negative correlation between As and K ($r = - 0.73$, $P<0.05$), and a strong negative correlation between V and K ($r = - 0.74$, $P<0.05$).

The content of B, Cu, Zn, Mn and Fe in processing tomato fruits did not differ significantly between the AMF+ and AMF- treatments in the present study (Figure 2). Pieper and Barrett (2009) reported that different cultivation and fertilization regimes, combined with AMF, had no impact on Cu, Fe and Zn content of processing tomato fruits as well. However, it is interesting to mention that the Fe content of analyzed samples ranged between 124.67 and 146.46 mg/kg (Figure 2), while Olaniyi et al. (2010) have reported the same nutrient in the range between 15.36 and 31.55 mg/kg. Even if a clear association between higher Fe soil and fruit content is not evident from the presented data, reported results may be linked to increased Fe availability in the *Terra rossa* soils in overall (Durn, 2003).

Concerning the trace elements content in the tomato fruit, application of AMF+ treatment has increased As and V and decreased Pb content (Figure 3). PLS analysis confirmed the results obtained by Tukey's test, where depending on the type of mycorrhization, the most distinguishing variables are Pb, As, and V (Figure 5). The obtained patterns could be related with the *Terra rossa* soil characteristics (Durn et al., 1999), interaction between tomato and mycorrhizal species (Janoušková et al., 2006; Kelly and Bateman, 2010; Bressy et al., 2013), and fertilization decisions (Elia et al., 2006; Kelly and Bateman, 2010; Bressy et al., 2013; Gupta et al., 2014). Reduced content of Pb in the mycorrhizae-inoculated treatment (Figure 3) could be a significant factor in processing tomato production, as according to EFSA (2012) in the European diet the major contributor of Pb, in the fruiting vegetables group, is tomato.

There are many factors that could increase the uptake of As, such as utilization of mineral phosphate fertilizers (Gupta et al., 2014), competition or replacement between As and other elements (Campos, 2002; Caoa et al., 2003; Gupta et al., 2014). Generally, it is known that mycorrhizal species act as buffers that control the metal level of their host plants (González-Guerrero et al., 2016). Additionally, difficulties related with As laboratory analysis led to

the absence of known As maximum levels in fresh or processed tomato fruits (European Commission, 2019). Burló et al. (1999) evaluated that the maximum content limit of As defined on dry weight (DW) basis in tomato fruits reaches up to 10 mg/kg. On the other hand, reported levels of V are very variable in the literature and range from 0.33 mg/kg FW (Byrne and Kosta, 1978) to 8.6 mg/kg DW (Anke, 2004) in tomato fruit, spinach 35 mg/kg FW (Byrne and Kosta, 1978), wild mushrooms up to 2000 mg/kg DW (Byrne and Kosta, 1978). According to EFSA (2006) the daily intake of V is limited to 2 mg/kg body weight/day.

It is interesting to mention that, of all analyzed trace elements, Li had the highest content with values between 63 mg/kg (non-inoculated) and 58.61 mg/kg (mycorrhiza-inoculated) treatments (Figure 3). Shahzad et al. (2017) reported Li content of 9.3 mg/kg in sampled tomato fruits. The same group of authors reported that chard Li content exalted in 197 mg/kg. Possible causes of observed Li content in our study are not clear from the reported data. However, such results showed that processing tomato fruits could be a significant natural source of Li in the food chain (Figueroa et al., 2013; Shahzad et al., 2017).

Total mycorrhizal colonization of tomato roots ranged between 79.65% and 90.65% (Figure 4), which is higher but comparable with some other open field tomato experiments (Bakr et al. 2017, Bakr et al. 2018). Inoculated plants in our study had initial advantage compared to non-inoculated control by having certain extent of established arbuscular mycorrhizae prior to planting in the open field as a good base for coping with the various stress. However, indigenous AMF communities in the open field soil were active and infectious for the control plants and although their total colonization was significantly lower than in inoculated plants, it reached at the end of the experiment very high values (79.65%) (Figure 4). These findings are similar to the ones reported by Ortas (2012).

CONCLUSIONS

Results of the 1-year long experimental study presented in this paper indicate that processing tomato can be highly susceptible to colonization both by selected and indigenous arbuscular mycorrhizal fungal species. However, even if the results show that mycorrhizal inoculation increases P and total phenolic content (hydrophilic fraction on fresh weight basis), benefits of such application are insufficient for commercial processing tomato production in the investigated conditions.

Among the analyzed trace elements in this study, Pb significantly decreased while As and V significantly increased in the fruits of mycorrhizae inoculated processing tomato plants. Even if increased in their concentrations, As and V pose no risk for human consumption due to their low concentrations determined in tomato fruits.

It remains to be further investigated what kind of interactions take place between the indigenous and commercial mycorrhizal strains and if they differ in efficiency of nutrient and water exchange with processing tomato plants. Finally, AMF could find a cost-effective application in commercial processing tomato productions when management decisions are arranged accordingly.

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