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# Environmental sustainability fruit quality and production in mycorrhizal tomato plants without P fertilizing

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**Abstract.** The influence of root colonization by arbuscular mycorrhizal (AM) fungus *Funelliformis mosseae*, on fruit quality, production and environmental sustainability were evaluated in field-tomato plants grown exposed to P-limited soil 5 µg $g^{-1}$ soil (basal-soil) with nitrate fertilization (50 µg $g^{-1}$ soil), after greenhouse germination and fungus colonization. After 60 days sowing (DAS), when the percentage of mycorrhizal root length (% RLC) raised at about 50%, the plants were transplanted in open field.
During the experiment, the mycorrhization has affected a lot of physiological aspects like vegetative and reproductive growth, improving them and ended the fruiting with a major fruit production that was 50% higher than not mycorrhizal (NM) plants. The ripening process of the fruits was also followed by testing sugars content and ß-Amylase activity in fruits of NM and mycorrhizal (M) plants fruits. At 140 DAS, in the harvesting fruits stage, fruits of M plants showed significantly higher mineral nutrient sugars and organic nitrogen compounds as amino acids and protein, compared to fruits from NM plants. In particular, GLU-GLN-ASP and ASN raised about 35% more than fruits from NM plants, improving nutritional aspect and flavor of the product. THR-ILEU-LEU-VAL and LYS, essential amino acids in man nutrition, increased around 25% more than fruits from NM plants, too. In this contest, lycopene, total carotenoids, ascorbic acid and glutathione (GS) and reduced form (GSH) were also tested in ripe fruits. The overall results suggest that tomato roots colonization by mycorrhizal fungus *Funelliformis mosseae* affects host plant nutritional status, modifying reproductive behavior, fruits production and nutritional quality.

**Key words:** *Funelliformis mosseae,* mineral nutrients, amino acids, mycorrhizal plants, *Solanum lycopersicum.*

## INTRODUCTION

The tomato *(Solanum lycopersicum* Mill.) fruit is one of the most popular, as well as one of the most important food, of the Mediterranean gastronomic culture based

primarily on quality and nutritional power. Over 40 million metric tons of tomatoes are produced each year on a world basis. The tomato is also popular because it grows well practically everywhere, as well as in the home garden and it preserves the nutritional quality in many dishes both raw and cooked. For its important role in the food market, tomato it was chosen as the ideal specimen for this investigation. The lack of typical flavor in supermarket tomatoes is a frequent complaint from consumers. Flavor is a composite of taste and odor (aroma), a relation exists between the kind of taste that a substance has and its chemical constitution as organic acids, inorganic salts, sugars, amino acids, long chain organic substances etc. Fruit aroma generally comes out by various volatile substances such as esters, aldehydes, ketones, and alcohols, which exist in small quantity in the fruit (El Hadi et al., 2013). The quality of a food product is also defined by its nutritional composition and active substances content, which are not less important than the organoleptic properties. In this contest the mineral and sugars concentration the amino acids pattern, the antioxidant concentration, as lycopene, GDH, carotenoids, vitamin C, citric acid etc., are good indicators of quality and value of the product. Fruit quality and productivity are the result of the environmental conditions and soil fertility (Brunetto et al., 2015). Nowadays, in particular in the Mediterranean area, land for agriculture, are a precious commodity in constant diminution. Therefore, it is essential that these areas can continue to be sustainably cultivated and that the fertility of their land remains intact. It is well knowing that tomato plants growth and reproduction they are greatly affected by phosphorus deficiencies, a condition of many agricultural lands (López-Bucio et al., 2002; Güsewell, 2004). Under these conditions, the colonization of plant roots by symbiotic fungi is highly advantageous (Cavagnaro et al., 2005). AMF are obligate biotrophs, which can form mutualistic symbioses with the roots of around 80% of plant species (Giovannetti, 1980) growing in nursery or field. It is well known that AMF symbiosis can increase plant growth and nutrient uptake, improving yield and fruit quality. The hyphae of AMF penetrate roots and grow extensively between and within living cortical cells, forming a very large and dynamic interface between symbionts (Farahani et al., 2008). The extraradical mycelia, instead, disperse outside the roots to have access to a greater quantity of water and soil minerals for the host plants. the colonization of the fungus and its influence on plant growth are strongly influenced by the mineral component in the soil (Grant et al., 2005). It is well known that the phosphorous soil content affects mycorrhizal development (Jeffries et al., 2003). In *Lotus japonicus* and *Medicago trunculata* selective P carriers, indispensable for phosphate transport through the plant cell arbuscular membrane, are also needed for mycorrhiza development (Harrison et al., 2002; Javot et al., 2007). The widest development of mycorrhizal colonization occurs when soil P concentration is suboptimal for plant growth, but it is often greatly reduced when soil P is largely available (Balaz & Vosatka, 1997), maximizing the use of inorganic fertilizer and the environmental sustainability between plant and soil. The purpose of this investigation was to establish the influence of mycorrhizal colonization on tomato fruit quality and productivity in P-limited soil. In particular, the roots mycorrhizal index and the fruit concentrations of mineral nutrients, total sugars, protein, free amino acids, lycopene, ascorbic acid and glutathione in oxidized were evaluated and reduced form.

## MATERIAL AND METHODS

### Experimental design

The tomato seeds (*Solanum lycopersicum* L. Desf cv Quorum F1) were separated in two groups: one group (M) was inoculated with spores of the mycorrhizal fungus *Funelliformis mosseae*, (Gerd. and Trappe) while the other one (NM) was not inoculated and used as control. Dry spores of *F. mosseae*, were kindly provided by the 'MS Biotech Spa', (Larino, Campobasso-Italy) in special packs of 100 g on inert powder support. The distribution of the spores in plastic pots (1.2 L) with soil mixture filled with 800 g DW (dry weight) of sterilized (120 °C for 30 min) medium-textured soil [clay : peat : sand 40 : 40 : 20 (v/v/v)] and the germination of the seeds took place in the climatic chamber as best reported by Di Martino et al. (2019).

After 60 days sowing (DAS) May (2016), when the percentage of mycorrhizal root length (% RLC) raised about at 50%, the plants have been transplanted in open field (locality Montefalcone (BN) Italy, elevation 700 m. A low P content soil (5-µg $g^{-1}$soil) received 90 kg ht ($KNO_3$) but no P fertilization. To promote the plant engraftment and to compensate the water losses by evaporation, 0.5 liters of water were administered every two days near the plants for the first six days long. Subsequently and until to the end of the experiment (140 DAS), the irrigations were carried out twice a day (at the - 7 a.m. and at the - 7 p.m. for 20 min) - with clear water by drip irrigation. The extension of radical mycorrhization, expressed in percentage of mycorrhizal root length (% RLC), were determined starting from the 28 DAS (the beginning of development and vegetative growth), until to 60 DAS in environmental chamber and successively in open field, until when the harvesting stage correspond in the present experiment at 140 DAS.

To ensure measurement homogeneity, a random number of generator was used to select five plants in each single particle (NM and M plants) (Stefan et al., 2013).

### Samplings, plant growth and mycorrhizal colonization analyses

The experimental design envisaged samplings from 21 DAS which is the beginning of development and vegetative growth, to 140 DAS, (harvesting stage, in the ours experiment), at increasing time intervals of two to four weeks. At each sampling, 3 NM and 3 M plants were randomly selected. The three plants were collected and used to be analyzed: the roots were washed with deionized water, dried through paper towels and promptly used for mycorrhizal colonization analysis. At the last sampling (140 DAS), part of the sampled roots were grounded in liquid $N_2$ to fine powder and stored at -40 °C to be used for elemental analyses. The mycorrhizal colonization was determined as reported by Di Martino et al. (2019).

### Free amino acid and protein analyses in fruits

Sixty fruits as sample (collected on five plant selected by random number generator) at 140 DAS (fruits harvesting stage) from M and NM plants, were washed, cut into pieces and seeds discarded. Fruits pieces were then homogenated by minipimer for 5 minutes in ice bath. The homogenized samples were held at -20 °C until analysis.

Drying of 10 g of homogenate in a flow of nitrogen in falkon tubes was solubilized according to Amalraj et al. (2010) with a solubilization buffer consisting of 7 M urea, 2 M thiourea, 2% CHAPS, 50 mM Tris- HCl, pH 8 and left at room temperature for

30 min. Tubes were centrifuged at 14,000 g for 10 min and the supernatant collected and used to determine protein and primary amino acids concentration.

The protein were determined by the Lowry method, using BSA as a standard and expressed in mg $g^{-1}$ FW (Lowry et al., 1951).

The free amino acids in the fruits were determined as reported by Di Martino et al. (2003 and 2006).

**Total sugars assay**

Refractometric methods was used to obtain only the total amount of sugar 20 g of homogenate (see above) were centrifuged at 15,000 g for 15 min. The total sugars content of supernatants were assayed using a refractometer HI96801 Hanna Instruments.

**ß-Amylase activity in fruits**

For the enzyme activity raiting, five fruits for sample (collected by random on five different plants (one for plant) from M and NM plants, of the three stages of fruits development were washed, cut into pieces and seeds discarded. Fruits pieces were then crushed into mortars by liquid nitrogen and mixed at a 1:1 (w/v) with an extraction medium as proposed by Di Martino et al. (2019) with some modifications. The extraction buffer contained 50 mM Tris HCl pH 7.5, 15% of glycerol (v/v), 0.25% Triton X-100 (w/v) and 20 mM $Na_2SO_3$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM phenylmethylsulphonylfluoride, (PMSF). 4% PVP-40 (Sigma Aldrich USA) The homogenate was filtered through four layers of muslin and centrifuged at 15,000 g at 4 °C for 15 min. The protein content of supernatants was determined using the Lowry method (Lowry et al., 1951), before the enzyme activity tests. The protocol Betamyl-3 (Magazine Wicklow, Ireland) was used for the determination of beta amylase activity according to the company instructions. The produced p-nitrophenol was assessed photometrically at 400 nm in a Jasko V-570 spectrophotometer.

**Determination of mineral elements concentration in fruits and soil**

5 g of NM and M fruits homogenated (see above) were oven dried for a week at 60 °C and ground to powder with a mortar and pestle; 0.1 g of each sample were then transferred into 25 mL beakers. Mineral elements were extracted by digestion of dried homogenate. The mineral elements content in the fruits as well as in the soil were determined as reported by Di Martino et al. (2019).

**Lycopene and total carotenoids determination in fruits**

About 400 g of fruits homogenate for each samples were lyophilized by vacuum freeze drying for 48 h. Lyophilized samples were used to carotenoids extraction according to the method reported by (Pintea et al., 2003 and Panfili et al., 2004).

**Ascorbic Acid determination in fruits**

1 g of fruits lyophilized sample (see above) was extracted with 3 mL of 5% *meta*-phosphoric acid. The homogenate was centrifuged at 10,000 g for 10 min at 4 °C and the supernatant was collected for the analysis of AA total by HPLC reverse phase in according to the method reported by Szalai et al. (2014).

**Glutathione determination in fruits**

Reduced (GSH), oxidized (GSSG) and total glutathione (GSH+GSSG) were determined according to Bashir et al. (2013). Five fruits for sample (collected on five plants (one for plant) selected by random number generator) from M and NM plants, were washed, cut into pieces and seeds discarded. Fruits pieces were then crushed into mortars by liquid nitrogen and to the powdered 5 mL of of 5% sulfosalicylic acid. The homogenates were centrifuged at 15,000 rpm for 15 min at 4 °C. n. For GSH determination, aliquots of supernatant (100–200 μL), were added to the reaction buffer (50 mM Na-phosphate pH 7.00, 0.3 mM EDTA and 0.005% 5,5-Dithiolbis 2-nitrobenzoic acid) in a final volume of 1,150 mL. The mix of reaction was read at 412 nm after 5 min. To the same, were added: 0.02% NADPH and glutathione reductase; 0.001 enzyme unit in a final volume of 1,200 mL. The reaction was run for 30 min at 25 °C. The samples were again read at 412 nm to determine the total glutathione. The values of glutathione concentration were determined against GSH standard curve (10–180 nmol). The used extinction molar coefficient was calculating to be 0.017 $cm^{-1}$ $nmol^{-1}$. The amounts of GSH and GSSG were converted and expressed in μg $g^{-1}$fw.

## RESULTS AND DISCUSSION

**Free amino acids and protein concentration in fruits**

The influence of mycorrhizal fungus on fruits pathways has been highlighted also by the increase of amino acids and protein abundance compared to the fruits control, although the changes of amino acid pattern in the fruits is also strongly influenced both by the stage of ripeness (Savioli et al., 2012) and the metabolic state of the leaf as source site of metabolite intermediates and precursor molecules for the fruits. In M red fruits at the ripeness, glutamate, glutamine, aspartate and asparagine, represent about 80% of total free protein amino acids in tomato fruits with increase of 20% of total protein on NM red fruits (Fig. 1). In particular glutamine, glutamate, aspartate and asparagine, raised the 35%, 35%, 38% and 40% respectively most of NM fruits. Moreover, a significant increase of alanine 27%, glycine 36% and cysteine 38%, has been registered on the NM fruits with enhancement of 20% total amino acids. Fundamental amino acids as threonine, isoleucine, leucine, valine and lysine also increased in the M fruits, with enhancement of 25% over NM fruits. An interesting note is that among amino acids increased, in the first group cited; glutamate and aspartate impart organoleptic characteristics to the fruits, (Birch, 1987; Cagan, 1987, Bellisle, 1991 and Chaudhari et al., 2000) improving the quality and nutritional, characteristics including the metabolic support for the human gluconeogenesis (Brosnan, 2003) whereas in the second group, glycine and cysteine, as response to oxidative stress represents essential substrates to glutathione pathway (Walquíria et al., 2017).

A glucogenic amino acid is an amino acid that can be converted into glucose through gluconeogenesis The production of glucose from glucogenic amino acids involves these amino acids being converted to alpha keto acids and then to glucose (Nelson & Cox, 2004).

As just said, the fruits amino acids pattern can improve both nutritional than organoleptic property. Studies of organoleptic characteristics of pure amino acids show that various amino acids can be described as being sweet, sour, salty or bitter. Solms (1969) and Stapleton et al. (1999) reported that L-glutamic acid and L- aspartic acid have a unique taste-potentiating characteristics and the participation of amino acids to aroma of foods often surmount the taste properties of the pure products.

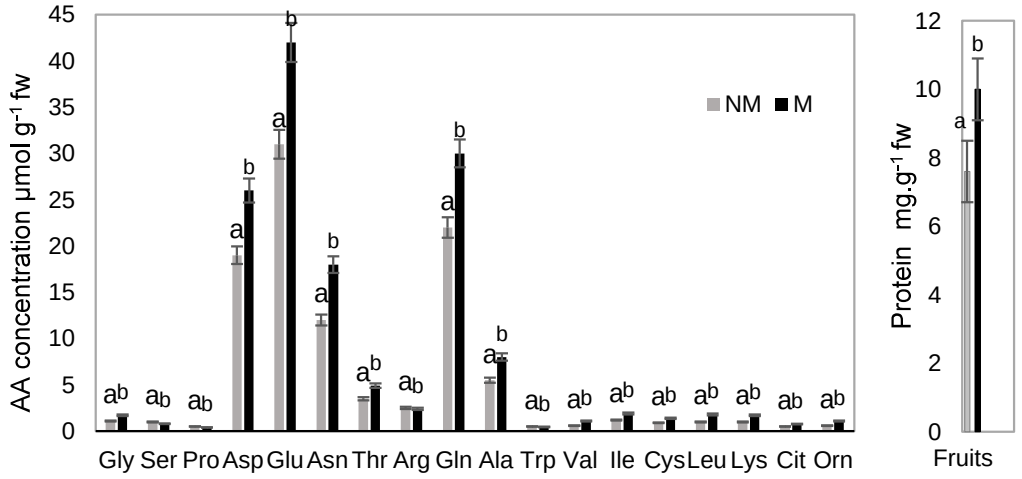


**Figure 1.** Average of amino acid and protein concentrations in the fruits of NM (grey bars) and M (black bars) tomato plants at 140 DAS. The values are means ± SD of 5 replicates. Bars indicate the standard deviation from five replicates. For every amino acid, values marked by common letters (a, a) are not statistically different, values marked by different letters (a, b) are statistically differen at $P \leq 0.05$ according to Tukey's test.

**Sugars total and ß-Amylase activity in tomato fruits**

Because the total soluble levels, are an important marker of fruits quality, their fluctuation were detected during the ripening of M and NM tomato fruits in three steps of ripening: Green fruit (G), Breaker (Br) and Red Ripe (RR). The total sugar determined in all fruits examined showed an linear increase during the whole fruits ripening phase reaching 65 and 80% more than the initial stage of maturation for NM and M fruits respectively (Fig. 2, a). Moreover, at RR stage total sugars in M fruits were 30% more than NM fruits. Since the content of total sugars in the fruits during the ripening process is the result of starch hydrolysis and ß-Amylase is the key enzyme that initiates starch degradation in most plant tissues, the ß-Amylase activity was also determined in the three stages of maturation (Fig. 2, b). ß-Amylase activity in all tomato fruits examined showed a maximum of activity at Br stage followed by a decline at the RR stage. Unlike the control, the fruits of M plants showed a significant amylase activity, already from the first stages of ripening of the fruits, indicating an advance on the ripening process with respect NM fruits. Moreover the ß-Amylase level in the fruits control were higher in all stage examined compared to the control.

More specifically they raised the 140; 30; and 50% more then NM fruits in G; Br and RR stage respectively.

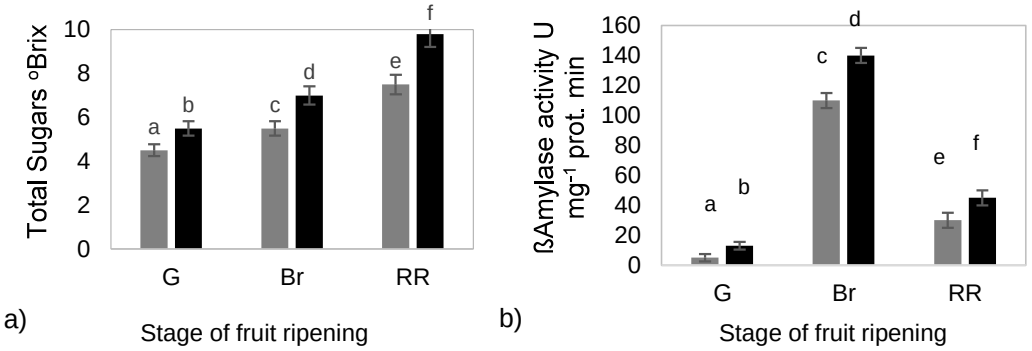


**Figure 2.** Total sugars content and ß-Amylase activity in NM (grey bars) and M (blak bars) tomato fruits during three stages of ripening process G (green fruits) Br (Breaker stage) RR (Red Ripening stage). Specific activities were expressed in $U^{TM}$ $min^{-1}mg^{-1}$ protein. The values are means ± SD of 5 biological replicates. Values marked by common letters are not statistically different at $P \leq 0.05$ according to Tukey's test.s

**Mineral elements concentration in soil and in fruits**

Macro (P, K, Ca) and microelements (Fe, Zn, Mn) concentration, in soil and fruits, respectively of M and NM plants were determined at 140 DAS (fruits harvesting stage) and shown in Table 1, at the step when root colonization was at the highest extent (Table 2).

**Table 1.** Fruits macro (P, K, Ca) and microelement (Fe, Zn, Mn) concentrations in soil and in not mycorrhizal (NM) and mycorrhizal (M) tomato plants at 140 DAS. The values are means ± SD of 4 replicates. Values marked by common letters are not statistically different at $P \leq 0.05$ according to Tukey's test performed between NM and M plants for the same treatment

| SOIL | N: ($NO_3$ 50 µg $g^{-1}$soil) + ($NH_4$ 5 µg $g^{-1}$soil) | | | | | |
|---|---|---|---|---|---|---|
| µg $g^{-1}$soil | [P] | [K] | [Ca] | [Fe] | [Zn] | [Mn] |
| Complete elements | 240 ± 18 | 2,600 ± 330 | 2,200 ± 160 | 900 ± 80 | 70 ± 8 | 600 ± 55 |
| Bioavailable elements | 4 ± 0.5 | 70 ± 8 | 27 ± 4 | 12 ± 1 | 4 ± 0.5 | 8 ± 1 |
| FRUITS µg $g^{-1}$dw | [P] | [K] | [Ca] | [Fe] | [Zn] | [Mn] |
| NM | 1,500 ± 250b | 2,600 ± 250a | 1,500 ± 150b | 40 ± 5b | 18 ± 2b | 17 ± 2b |
| M | 2,200 ± 300a | 2,800 ± 250a | 2,000 ± 200a | 70 ± 5a | 30 ± 3a | 25 ± 3a |

Table 1 shows that macronutrient and micronutrients concentration in tomato fruits was higher in M compared with NM plants in which no significant fungal colonization was found. In particular, for macronutrients, P and Ca, and micronutrients Fe and Zn, there were significant variations between M and NM plants (about 45% and 70% increase respectively). Conversely, there were not significant difference between M and NM plants in about K fruits concentration. In particular the absolute concentration in mycorrhizal tomato fruits for P, Ca, Fe and Zn, 2,200, 2,000, 70 and 30 µg $g^{-1}$dw fruits

respectively compared with soil content 5, 25, 15 and 5 μg $g^{-1}$soil are in according to the vision that mycorrhizal roots, by means the fungal hyphae, can more widely explore the rhizosphere and catch mineral nutrient, also through an higher nutrient uptake capacity and accumulation in the plant tissues with respect to the not mycorrhizal ones. Because nutrient concentrations in the fruits generally followed the same trend as those in the plant, an enhanced uptake of mineral nutrients from soil, may correspond to an enhancement of mineral concentration in the fruits.

**Table 2.** Mycorrhization index (% RLC). Mycorrhization Index (% RLC) at increasing time intervals of two to four weeks of M and NM plants during growth in P-limited soil (5 μg $g^{-1}$soil) The values are means ± SD of 4 replicates. For every (% RLC), values marked by different letters (a, b) are statistically different at $P \leq 0.05$ according to Tukey's test

| DAS | 21 | 35 | 56 | 84 | 112 | 140 |
|---|---|---|---|---|---|---|
| NM (%RLC) | 3 ± 1a | 6 ± 2a | 7 ± 2a | 6 ± 2a | 7 ± 2a | 5 ± 1a |
| M (%RLC) | 12 ± 3b | 35 ± 5b | 50 ± 5b | 52 ± 6b | 55 ± 5b | 58 ± 6b |

In fact, in the fruits the macronutrients as well micronutrients concentrations were higher in M compared with NM plants, in particular, P, Ca, for macronutrients, Fe, and Zn for micronutrients that improve the nutritional quality of the fruits. Calcium and phosphate supplements in aliment diet, support proper cellular signaling, proper nerve conduction (Sundelacruz et al., 2019) and a decreased risk of same decreases. Iron, instead present, in red cells is essential for carrying oxygen from the lungs to the body's tissues and in mitochondria in electron, transfer reaction of respiratory chain, zinc is needful for insulin and thyroid function (Baltaci et al., 2019) and collaborates to protect the body from free radicals.

**Carotenoids and antioxidants fruits concentrations**

For their antioxidant propriety, an increase of lycopene and carotene, in M fruits improve the fruits quality as well as the external appearance providing greater appreciation of the product, in fact, though glutathione as well ascorbic acid (vitamin C) exercising an important influence as an antioxidant and defendes the plant during oxidative damage by scavenging free radicals and ROS (Schulthesis et al., 2002; Elwan & El-Hamahmy, 2009), to a lesser extend, also carotenoid have ROS scavenging activity and the most potent antioxidant among carotenes is lycopene, which imparts also red color to the tomato fruit.

The data in Table 3 shows the Lycopene, total carotenoids, Glutathione and vitamin C concentrations of M fruits compared with NM fruits.

**Table 3.** Antioxidant substance concentrations in NM and M tomato fruits. Carotenoids, ascorbate and glutation concentration in tomato fruits collected at 140 DAS. The values are means ± SD of 6 replicates. Values marked by common letters are not statistically different at $P \leq 0.05$ according to Tukey's test performed between NM and M plants for the same treatment

| Red Fruits | Lycopene μg $g^{-1}$FW | Tot. Carotenoids μg $g^{-1}$FW | Ascorbate μg $g^{-1}$FW | GSH μg $g^{-1}$FW | GS μg $g^{-1}$FW |
|---|---|---|---|---|---|
| NM | 50 ± 7b | 65 ± 7b | 170 ± 15a | 50 ± 6a | 20 ± 3a |
| M | 70 ± 8a | 90 ± 9a | 190 ± 18a | 55 ± 7a | 22 ± 3a |

In M fruits, the lycopene concentration in harvesting stage was 45% higher than NM fruits supported the importance and the function that mycorrhizal fungi have particularly to potentiate the nutritional and nutraceutical quality of tomato fruits through changing of plant secondary metabolism that led to amplify levels of lycopene in fruits obtained from mycorrhizal plants (Giovannetti et al., 2012). In the same tendencies, the value of total carotenoids in the fruits was increased by 40% with the treatment of mycorrhizal fungus over the control (Salem M. Al-Amri, 2013). On the contrary, the same table shows that there were no significant variation in ascorbic acid and glutathione concentration between M and NM plants. The indication that antioxidant compounds as ascorbic acid and glutathione, can maintain in tomato fruits of M and NM an relatively low level, can suggest an low oxidative damage to the biomolecules present in plant systems involved and that mycorrhizal symbiosis in this context does not influence the antioxidant metabolic pathways. In the other hands, an enhancement of their metabolic precursor in treated plant fruit as: cysteine, glycine and glutamate, can prepare the cell to earlier GSH synthesis by stress input (Liu et al., 2018).

**Fruiting, fruits ripening and production**

M tomato plants also showed an earlier fruiting and fruits production increase. The mean of fruiting date (defined as the date at which 50% of the plants had produced their first fruits) was substantially anticipated in the mycorrhizal condition and occurred at 98 DAS, while the NM plants reached fructification about one week later (Fig. 3). Also the differences in fruits ripening date between NM and M plants, showed the same shift of fruiting date. Indeed, the time needed to reach the red stages was 7 days shorter for the M plants (126 DAS on 133 DAS) compared to NM plants.

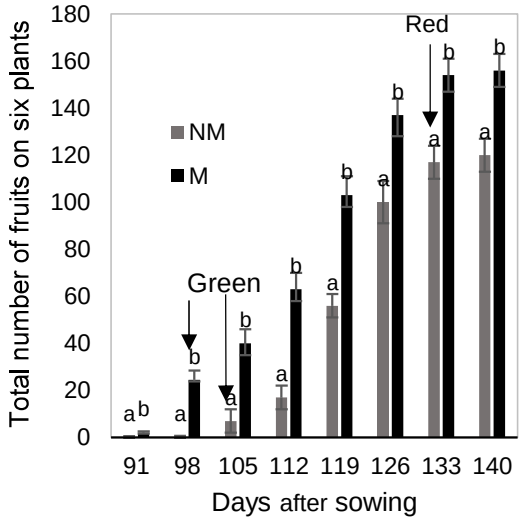


**Figure 3.** Fruiting evolution, detected as average of the total number fruits present on five plants every week during the reproductive phase in NM (grey bars) and M plants (black bars). The arrows indicate the beginning of fruiting and fruits ripening. The values are means ± SD of 3 replicates. Values marked by common letters are not statistically different at $P \leq 0.05$ according to Tukey's test performed between NM and M plants for the same treatment.

The mycorrhization of *Funelliformis mosseae* increased significantly also the average of fruits number, and fruits weight of mycorrhizal tomato plants compared with control plants: about 155 and 120 fruits, respectively were harvested at 140 DAS as a total amount from five plants and averaged in three replicate (Table 4).

The average of the amount fruits weight harvested from five M plants as above mentioned also increased on the control: 1,700 g and 1,080 g respectively. This led to an increased fruits productivity of the M plants of about 50% on control.

Furthermore, evident differences also were observed for the average of individual fruit weight (of the five plants) 11 and 9 g for M and NM plants respectively in the harvesting stage (140 DAS). (Table 4). Our findings confirm those of other studies as the results of Copetta et al. (2011) who decleared that root mycorrhization induced an increase in growth and number of fruits of a different tomato variety.

**Table 4.** Fruits production. Average of the total number and total weight fruits harvested from five plants at 140 DAS. The values are means ± SD of 3 replicates. Values marked by common letters are not statistically different at $P \leq 0.05$ according to Tukey's test

| Numerical average of fruits collected from five plants | NM | M |
|---|---|---|
| Numerical average of total fruits from five plants | 120 ± 6b | 155 ± 8a |
| Numerical average of total fruits weight from five plants | 1,080 ± 70b | 1,700 ±110a |
| Numerical average weight of one fruit (g) of a100 examined | 8 ± 0.6b | 11 ± 0.7a |

At large extend the ontogenetic cycle of the plant was also favorably affected by mycorrhization. The control plants showed a progressive loss of vigor and half of them died approximately five months after transplanting, whereas the mycorrhizal plants continued to produce fruits for at least five more months (data not shown).

The tomato plants favoured mycorrhiza symbiosis with *Funelliformis mosseae* in soils with phosphorous content 5 μg $g^{-1}$soil (Table 2), in agreement with the findings that mycorrhizal development is affected by plant nutritional status (Baum et al., 2002; Treseder et al., 2004). The mycorrhizal index (% RLC) reaching 50% at 56 DAS.

The mycorrhization of *Funelliformis mosseae* greatly affected the growth parameters and flowering (data not shown). But in mycorrhizal plants, these alteration of the morphometry also influenced the reproductive growth.

In fact, the mycorrhizal plants being more developed reached the reproductive phase early. Hildebrandt et al. (2002) reported that a treatment of *Glomus intraradices* accelerates the change from vegetative to reproductive phase. the reason could be attributed to the improvement of the nutritional status that positively influences the transition of vegetative meristem to reproductive meristem in tomato, (GU et al., 2010) have identified a group of microRNAs in tomato leaves, which is exclusively induced by AM symbiosis.

The mycorrhizal plants showed a significant advance of flowering, of about ten days on not treated plants, which ended also an early fruiting.

The most interesting evidence obtained in this study was that the mycorrhizal plants, have shown significant increases in the flowering (data not shown) followed significant increases in the tomato fruits number with about 40% more of fruits production compared with no treated plants (Table 4).

The earlier and more pronounced fruiting phase in M plants might result from enhanced capture of mineral nutrients from soil, chiefly immobile ions like P, Ca and Zn, and improved nutrient translocation system that may correspond to an enhancement in yield and mineral concentration in the fruits.

Utkhede (2006) indicated also that M tomato plants produced significantly higher fruits number and weight fruits production compared to the NM plants.

The influence of mycorrhizal fungus on fruits pathways was also evidenced as increase of sugars and amino acids abundance compared to the fruits control, in particular the total soluble solid was detected in three steps during the fruits ripening

showing an increase from initial phase (G) to maturity (RR) of 50 and 70% for NM and M fruits respectively.

This trend proceeded with a parallel increase of ß-Amylase activity which hydrolyses starch, increases sugar metabolic levels.

In particular, in NM fruits the substantial lower activity of the enzyme at G stages suggests that ß-Amylase probably detain a more significant function in starch degradation during the late stages, of fruits development. In M fruits instead, significant ß-Amylase activity was already evidenced in early stages of ripening fruits increasing the sugar content already from the first stages of maturation. Moreover, in M fruits ß-Amylase maintaining higher activity levels than NM fruits in all three stages of ripening fruits detected confirming a close relationship between ß-Amylase activity and sugar content in the fruits.

A greater boost to the maturation process in the M fruits can arise from the positive role of ABA in triggering ethylene biosynthesis and ripening of tomato fruits Zhang et al., 2009), furthermore the arbuscular-mycorrhizal (AM) fungus Glomus sp. produces ABA, and ABA concentration in the xylem sap is higher in mycorrhizal than non-mycorrhizal plants (Esch, 1994). In the other hands exogenous ABA accelerates fruits ripening, and fluridone or NDGA treatmentdelayed fruits ripening by inhibition of ABA (Esch, 1994). A greater resource of sugars in M fruits forms adequate carbon skeletons for synthesize a large fraction of primary and essential amino acids, in particular at 140 DAS before the harvesting, giving organoleptic characteristics and nutritional power to the product as well described above, glutamine, glutamate, aspartate and asparagine, raised the 35%, 35%, 38% and 40% respectively most of the control. A significant increase of alanine 27%, glycine 36% and cysteine 38%, was registered, with enhancement of 20% total amino acids. Essential amino acids as threonine, isoleucine, leucine, valine and lysine also increased in the fruits from M plants, with enhancement of 25% over control. An interesting note is that among amino acids increased, in the first group cited; glutamate and aspartate, as well as being indispensable substrates for uman gluconeogenesis, impart organoleptic characteristics to the fruits, improving the quality, whereas in the second group, glycine and cysteine, as response to oxidative stress represents essential substrates to glutathione pathway.

As just said, the fruits amino acids pattern, can improve both nutritional than organoleptic property. Studies of organoleptic characteristics of pure amino acids show that various amino acids can be described as being sweet, sour, salty or bitter. Solms (1969); Stapletonet et al., 1999; Kurihara et al., 2015) reported that L-glutamic acid and L- Aspartic acid have a unique taste-potentiating property and the contribution of amino acids to flavour of foods often exceed the taste properties of the pure compounds. The quality of a fruit is important crossroad between nutritional properties flavor and organoleptic characteristics that are often associated to the color and good appearance of the fruit.

In this contest, lycopene and carotene symbolize the principal carotenoids in tomato fruits that impart color, giving an initial perception of quality. In fact, those important nutrients as also used as a color ingredient in many food formulations, and their increase in M fruits of 45% and 40% of lycopene and carotene makes the fruits more palatable.

For their antioxidant propriety, an increase of lycopene and carotene, in M fruits improve the fruits quality as well as the external appearance providing greater appreciation of the product. Inversely, there was not found difference in ascorbate and glutathione concentration between NM and M plants. For their significant antioxidant characteristic those findings, indicating a low oxidative damage to the biomolecules present in the fruits. In the other hands, an enhancement of their metabolic precursor in treated plant fruit as: cysteine, glycine and glutamate, can prepare the cell to earlier GSH synthesis.

Among the various effects caused, the mycorrhizal fungi could also interact with the training effects of the plant system on quality and productivity. In fact, the mycorrhizal plants had higher fruits yield per plant, moreover the red stages started 7 days earlier and fresh weight was 50% higher than that of the control. The enhancement in fruits yield per plant due to mycorrhizal inoculation might be attributed to enhanced photosynthesis associated with increased P uptake in plants (Dietz & Foier, 1986; Thuynsma et al., 2016), and hence high amounts of assimilates were likely produced to support both symbiosis and fruits development. The acceleration in the velocity of fruits development is possibly achieved because of the compatibility between *Funelliformis. mosseae* and the local ecotype of *Solanum lycopersicum* L that grew in the its natural ecosystem. These results show the importance of achieving a better association between a determined AMF strain and the ecotype of tomato, in order to improve and optimize the production process (Castillo et al., 2009).

## CONCLUSIONS

1. Mycorrhizal colonization in tomato plants has been highly extended in our experimental conditions.

2. The fruits nutrients concentrations of M plants were significantly higher than those of NM ones, and it supported also the view that mycorrhiza actively modulate nutrient uptake limiting their interferences and optimizing the growth had better than the plant own roots. Fungal hyphae behave like a very efficient rootstock in the given experimental conditions.

3. The mycorrhization of *Funelliformis mosseae* greatly affected fruits productivity and quality of tomato plants, grown in open field under P limiting conditions by increasing the sugars content and nitrogen metabolism in plants, as well as enhances fruits carotenoid and lycopene concentrations. The increase of mineral nutrients, protein and free amino acid concentrations among which, glutamate, aspartate as well as threonine, isoleucine, leucine, valine and lysine, essential amino acids in man nutrition, suggested that the M fruits tissues had a higher nutritional value and taste property than the NM ones.

These results indicate that the use of AM inoculum of *Funelliformis mosseae* in P-limited soil can improve fruits production and quality, of tomato plants affecting biochemical composition and relative proportion of various mineral nutrients.

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