

EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON TOMATO YIELD AND NUTRIENT UPTAKE UNDER DIFFERENT FERTILIZATION LEVELS

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Received: September 13, 2012

Abstract

NEDOROST, L., POKLUDA, R.: *Effect of arbuscular mycorrhizal fungi on tomato yield and nutrient uptake under different fertilization levels*. Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 8, pp. 181–186

Effect of the arbuscular mycorrhiza on tomato plants (*Lycopersicon lycopersicum*) in the pot experiment was studied. Three different fertilization regimes (optimum – H1, stress a – H2, stress b – H3) and three different mycorrhizal treatments (control – Ctrl, *Glomus mossae* – Gm, *Glomus intraradices* – Gi) were used. Economical parameter (yield), nutritional characteristic (vitamin C content, phosphates and minerals content, total antioxidant capacity), and level of root colonization were studied. The yield of the tomatoes was influenced by the basic dose of the fertilization, especially in the H2 and H3 treatment. The highest yield was in the H2 treatment in Gm (938 g per plant). The positive effect of the inoculation resulted in the increased content of the vitamin C. The highest significant influence was observed in the H2 treatment (plants inoculated with Gi) with the average content of the vitamin C 289 mg.kg⁻¹. The average rate of the colonization was in the range from 39% to 65%.

mycorrhiza, vitamin C, TAC, AMF colonization, tomato

A tomato is an important crop throughout the world and it is grown under a wide range of production systems. The tomato (*Solanum lycopersicum* L.) is very valuable from the perspective of nutritional value, for example it is a source of the different classes of the antioxidants such as carotenoids, ascorbic acid, phenolic compounds, and α -tocopherol (Beecher, 1998; Abushita *et al.*, 1997). This is the reason for searching of new opportunities to improve nutritional content in a tomato under lower level of the fertilization. The use of the arbuscular mycorrhizal fungi (AMF) is one of the possibilities. AMF is the most widespread root fungal symbiosis and it is associated with the vast majority of the higher plants. It represents a mutualistic symbiosis between AMF and the roots of terrestrial plants (Smith and Read, 1997). The fungi colonize roots of approximately 90% of the Earth's land plant species (Gadkar *et al.*, 2001). AMF improves the absorption of the several nutrients,

such as phosphorus (Harley and Smith, 1983; Al-Karaki and Al-Raddad, 1997; Chandreshekhara *et al.*, 1995), nitrogen, potassium, calcium and magnesium (Liu *et al.*, 2002), copper (Gildon and Tinker, 1983) and zinc (Faber *et al.*, 1990; Gildon and Tinker, 1983). AMF can increase content of the antioxidant in inoculated plants (Huang *et al.*, 2011; Talaat and Shawky, 2011). Arbuscular symbiosis can improve a soil structure and protect host plants against the detrimental effects caused by the drought stress (Schreiner *et al.*, 1997).

MATERIALS AND METHODS

Experimental site, plant and substrate materials

The pot experiment was located outdoors under the field conditions at the Faculty of Horticulture in Lednice (Location: 48°47'54.502"N; 16°48'0.39"E,

Czech Republic) in 2011. Seeds of *Lycopersicon lycopersicum* (Solanaceae) cv. "DARINKA F1" (SEMO a. s.) were sown on 21st March, and seedlings were planted into experimental 10 liter pots on 30rd May. Properties of substrate were: 65 % of dry matter, pH 5.8, the electric conductance of the substrate was 1.2 mS.m⁻¹ and the basic fertilization of the substrate (from producer) was PG-mix (N-P-K - 14:16:18) 1.5 kg/1000l. The substrate was fertilized, based on a chemical analysis, with 6.4 g of CaCO₃ per pot to achieve pH = 6.3.

Experimental design with microbial treatments

The experiment contained 54 containers with tomato plants under three different fertilization regimes (optimum – H1, stress a – H2, stress b – H3) and three different treatments (control – Ctrl, *G. mosseae* BEG95 – Gm, *Glomus intraradices* BEG140 – Gi.) 10g, 5g and 2.5g respectively of calcium sulphate (14% sulfur content) were added in H1, H2 and H3 fertilization treatment respectively. 10g, 5g and 2.5g respectively of bone meal (calcium phosphate content of 80%) were added in H1, H2 and H3 fertilization treatment respectively.

Microbial inoculum was supplied by the company Symbiom ltd. The mycorrhizal inoculum (Gm and Gi) was added as a mixture of the cultivation substrate, colonized roots and mycelium fragments (grown as single AMF cultures on a maize as the host plant for 5 months in zeolite) in a dose of 100 g per planting hole approx. 3 cm below each seedling. Each of the 3 mycorrhizal treatments and 3 fertilization regimes was composed of the 6 pot repetitions, each pot contained 1 seedling.

Harvests, plant analyses, mycorrhizal parameters

The plants were harvested, measured and sampled for the plant analyses in August 2011. Roots of all plants were sampled to determine AMF colonization.

Vitamin C (ascorbic acid) determination

The concentration of vitamin C (ascorbic acid) was determined by HPLC according to Arya *et al.* (2000) with slight modification. Tomato fruit samples were homogenized in a blender with 75 ml of 0.1 M oxalic acid. The homogenate was topped up with oxalic acid to the volume of 100 ml, filtered, centrifuged (3,800 rt/min for 10 minutes) and the supernatant was used for measurement. The analyses were performed by RP-HPLC in a LCO-101 column placed in an Ecom thermostat (t = 30 °C), mobile phase TBAH (tetrabutylammonium hydroxide): 0.1 M oxalic acid: water in the ratio of 10:20:70 (v/v/v), flow 0.5 ml/min at 254 nm using a UV-VIS detector. The amount of AA was expressed as mg.100 g⁻¹ of fresh weight.

Antioxidant capacity determination by FRAP assay

Tomato fruit samples (10 g) were homogenized in a blender with 30ml ethyl alcohol (50% concentration), homogenate was added with ethyl alcohol (50% concentration) to amount of 50ml, filtered, centrifuged (3,800 rt/min for 10 minutes), and the supernatant was used for the measurement. Total antioxidant capacity (TAC) was determined using the ferric reducing antioxidant power (FRAP) assay developed by Benzie and Strain (1996). In the FRAP assay, reductants ("antioxidants") present in the extract reduce Fe(III)-tripyridyltriazine (TPTZ) complex to its intensely blue ferrous form with an absorption maximum at 593 nm. The working FRAP reagent was prepared fresh on the day of the analysis by mixing acetate buffer, 10 mM TPTZ solution and 20 mM ferric chloride solutions in the ratio of 10:1:1 (v/v/v). The mixture was incubated at 37 °C. The absorbance was monitored for 4 min in a temperature-controlled cuvette held at 37 °C using a JENWAY 6100 spectrophotometer (AIR, UK). The final total antioxidant capacity was expressed in mg equivalent of Trolox in 100g of fresh biomass (mg Trolox.100g⁻¹).

Content of mineral elements, phosphates and sulphites

Content of Na, K, Ca, Mg, PO₄ and SO₄ were determined by the method of capillary isotachopheresis using the IONOSEP 2003 device (RECMAN, CZ) following method described by Blatny *et al.* (1997). Tomato samples (20 g) were homogenized and diluted with distilled water (1:40, v:v) and then analysed. The amount of each mineral element was expressed as mg per Kg of tomato fruit fresh weight (fw).

Mycorrhizal colonization

Mycorrhizal root colonization was evaluated in root samples taken from root systems of experimental plants. Ten roots fragments in long 10mm were observed from each plant. Samples were stained with 0.05% trypan blue in lactoglycerol (Koske and Gemma, 1989) and quantified by the modified grid-line intersect method (Giovannetti and Mosse, 1980) using an ocular grid at a 100× magnification.

Statistical Analysis

The statistical analysis was carried out in Statistica 9 program (StafSoft Inc.1984-2009) using the ANOVA and LSD methods at the significance level of P < 0.05. Measure of variability of a mean value throughout the text is expressed as standard deviation.

RESULTS AND DISCUSSION

First goal of the experiment was to find out if the tomato plants were significantly colonized with

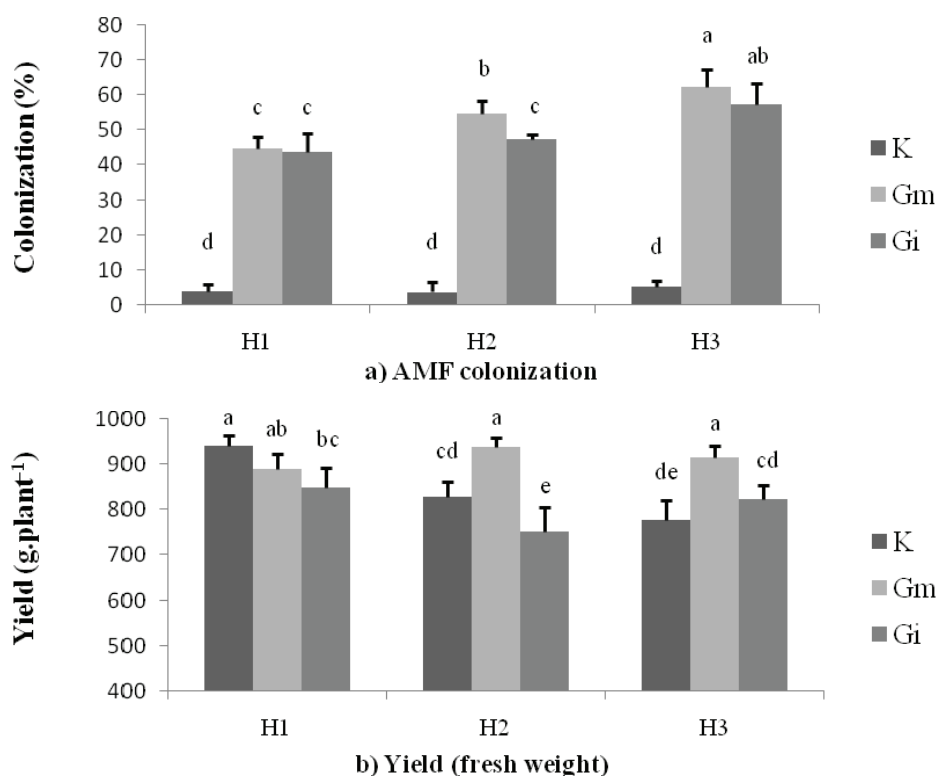
AMF (Fig. 1). It was expected that a commercial substrate contains only a little amount of AMF. This statement was finally confirmed. Plants from the control treatment had a rate of the colonization in the range from 3.8% (H1) to 5.4% (H3) in average. Plants from mycorrhizal treatment Gm had a rate of the colonization in the range from 44% (H1) to 62% (H3), and from treatment Gi in the range from 43% (H1) to 57% (H3). Karagiannidis *et al.* (2002) found similar levels of *Glomus* colonization in a tomato (49%). Higher level of colonization was found in plants which had a lower dose of the fertilizer. Significant differences were found between mycorrhizal treatments in the fertilizer level H1 and H3. A similar conclusion was reached by Hajiboland *et al.* (2009). He found that rice under optimal conditions of the phosphorus gave worse values of the colonization.

As it is shown in the Fig. 1, a fertilization level had the significantly positive effect on the yield especially on control plants. In the highest level, the yield was in Ctrl 941 g.plant⁻¹, in the fertilization level H2 829 g.plant⁻¹ only and in H3 777 g.plant⁻¹ only. The phosphor fertilization had the positive effect on a production of flower (Poulton *et al.*, 2001), what it was shown in the tomato yield. If the level of the fertilization was lower, the yield was lower as well. This negative effect was reversed with a help of the mycorrhizal fungi. The positive effect of the inoculation was found in the H2 and H3 treatment. The highest yield was in the H2 treatment in the treatment Gm (938 g per plant) in comparison to Ctrl

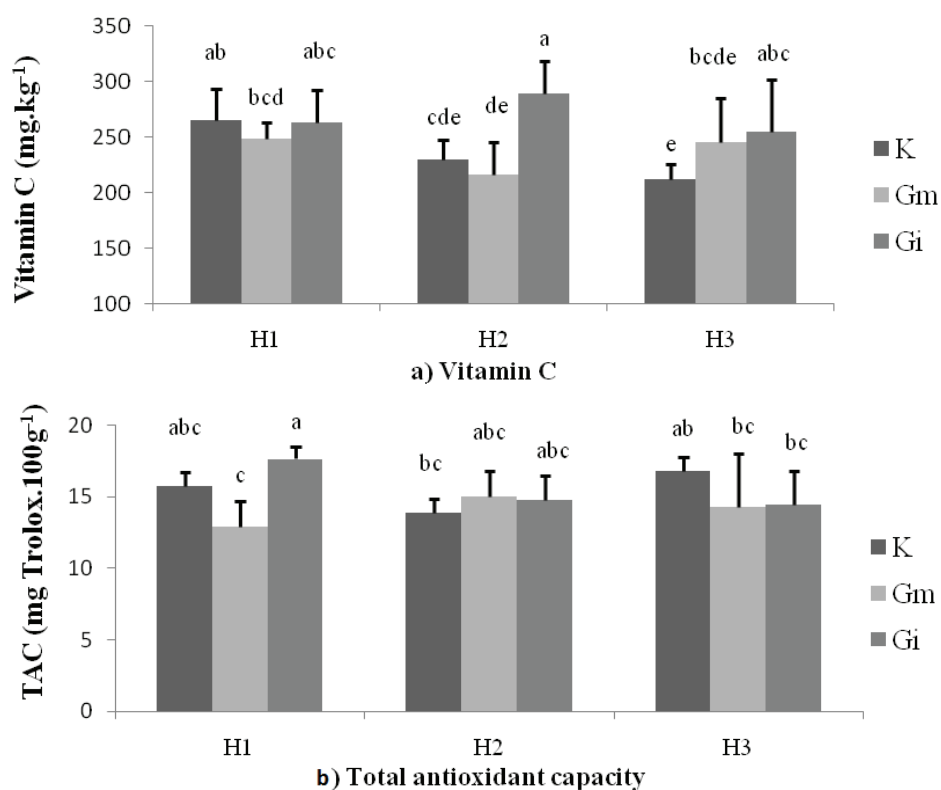
(838 g per plant), which makes an increase of 10%. In the H3 treatment, the highest yield was in the variant Gm (913 g per plant) in comparison to control (777 g per plant), with an increase of 14%. Hypothesis, that AMF can improve the yield, is corresponding with many other results, for example Al-Karaki and Clark (1999) or Valentine *et al.* (2007).

The highest content of vitamin C (ascorbic acid) was recorded in the variant H2 (Fig. 2), in the treatment Gi (289 mg.kg⁻¹), an increase of 20% in comparison to the Ctrl (230 mg.kg⁻¹). However, the second highest content (265 mg.kg⁻¹) of vitamin C was found in non mycorrhizal treatment Ctrl in fertilization H1. The content of vitamin C was decreased with the decreasing level of the fertilization. In the Ctrl treatment and H3 level of fertilization the lowest content of vitamin C (212 mg.kg⁻¹) was found. The microbial treatment was able to increase the content of vitamin C in a lower level of the fertilization H3 in comparison to Ctrl. The content of vitamin C was 16% (Gi) and 13% (Gm) higher than Ctrl. The ability to increase the vitamin C content by mycorrhiza describes Dey *et al.* (2005).

The positive effect of the inoculation on the antioxidant capacity was found in the variant with the highest dose of the fertilization mainly (Fig. 2). In the experiment, treatment Gi in variant H1 (17.6 mg Trolox.100 g⁻¹) had the best result, in comparison to Ctrl (15.7 mg Trolox.100 g⁻¹), with an increase of 10%. Huang (2011) also describes the positive effect of the mycorrhiza on the content of the antioxidants



1: a) AM colonization, b) Yield fresh weight. Means \pm SD, columns marked with the same letters are not significantly different at the level $P < 0.05$, LSD Test, $n = 6$



2: a) Vitamin C, b) TAC. Means \pm SD, columns marked with the same letters are not significantly different at the level $P < 0.05$, LSD Test, $n = 6$

in a watermelon. In the treatment H2, differences between treatments were not significant. In H3 variant, the second highest content of antioxidants in the non mycorrhizal plants was found ($16.8 \text{ mg Trolox.}100 \text{ g}^{-1}$). The content of the antioxidant in Gi was $14.4 \text{ mg Trolox.}100 \text{ g}^{-1}$ and in Gm $14.3 \text{ mg Trolox.}100 \text{ g}^{-1}$ only. According to our results, it seems there is a trend the mycorrhizal treatment Gi increases the content of antioxidants especially in the variant of higher doses of the fertilizer. The individual effect of the different fertilization may influence the content of the TAC on vegetables. Albrechtova *et al.* (2012) described that the application of the mycorrhiza can increase activity of antioxidants in an onion.

Another part of the experiment was determining the content of phosphates, sulphates and minerals in fruits. Results are showed in Tab I. The highest content of PO_4 in the fruit was found in H1 level of the fertilization and in Gi treatment, an increase in comparison to the Ctrl H1 represented 63%. The treatment Gm H1 had also very good result. The content of PO_4 was $1369 \pm 210 \text{ mg.kg}^{-1}$. It is significantly more than in Ctrl H1. In other levels of the fertilization H2 and H3, no significant differences were found between treatments. The significantly variance was found only between levels of the fertilization, the content of PO_4 was the highest with the highest level of the fertilization. The mycorrhizal treatment was able to increase the content of PO_4 only in the highest fertilization H1.

Many authors describe the ability of the mycorrhizal fungi to increase the content of P, for example Liu *et al.* (2007) or Valentine *et al.* (2007).

SO_4 analysis showed the positive effect of the mycorrhiza, mainly in the H1 level of the fertilization. Plants from the treatment Gi H1 and Gm H1 had the significantly highest content of SO_4 than plants in Ctrl H1. The significantly highest content of SO_4 in the variant H2 had also the treatment Gm in comparison to Ctrl H2. No significant difference was found between treatments in H3 level of the fertilization. There is not confirmed effect of the mycorrhiza on the content of SO_4 .

No stronger influence of the fertilization on the content of Ca was found, but the influence of the treatment was significant. The highest content of Ca was found in Gm treatment in average $173 \pm 12 \text{ mg.kg}^{-1}$ in comparison to Ctrl ($122 \pm 17 \text{ mg.kg}^{-1}$ in average), which makes an increase of 41%. In the treatment Gi ($154 \pm 5 \text{ mg.kg}^{-1}$ in average) was found also the highest content of calcium, in average 26% more than in Ctrl. The positive effect of the mycorrhiza to the content of calcium observed also Seleh *et al.* (2006). The positive influence of the mycorrhiza on maize was confirmed in his study.

No significant difference was found in the analysis of K, Na, Mg (Tab. I). The highest non significant different in the content of K was found in the treatment Gi H3. In the treatment Gm H1, the highest non significant content of Na was observed. The content of Mg was highest in the treatment

I: Means \pm SD, columns marked with the same letters are not significantly different at the level $P < 0.05$, LSD Test, $n = 6$, fw – fresh weight

microbial treatment	fertilization	PO ₄ (mg.kg ⁻¹ fw)	SO ₄ (mg.kg ⁻¹ fw)	K (mg.kg ⁻¹ fw)	Na (mg.kg ⁻¹ fw)	Ca (mg.kg ⁻¹ fw)	Mg (mg.kg ⁻¹ fw)
Ctrl	H1	995 \pm 136 b	450 \pm 66 b	1685 \pm 152 a	185 \pm 22 a	121 \pm 17 de	114 \pm 11 a
	H2	589 \pm 268 c	17 \pm 3 d	1773 \pm 201 a	167 \pm 24 a	130 \pm 11 cde	109 \pm 10 a
	H3	412 \pm 151 c	13 \pm 8 d	1573 \pm 73 a	185 \pm 18 a	117 \pm 25 e	113 \pm 11 a
Gi	H1	1626 \pm 226 a	671 \pm 31 a	1668 \pm 183 a	185 \pm 36 a	162 \pm 3 b	118 \pm 5 a
	H2	611 \pm 51 c	175 \pm 54 c	1592 \pm 131 a	175 \pm 15 a	143 \pm 2 bcd	107 \pm 5 a
	H3	436 \pm 32 c	56 \pm 68 d	1800 \pm 139 a	181 \pm 21 a	157 \pm 3 b	118 \pm 6 a
Gm	H1	1369 \pm 210 a	653 \pm 81 a	1774 \pm 76 a	192 \pm 17 a	211 \pm 8 a	120 \pm 6 a
	H2	503 \pm 35 c	462 \pm 50 b	1653 \pm 144 a	172 \pm 17 a	158 \pm 15 b	110 \pm 11 a
	H3	406 \pm 63 c	28 \pm 36 d	1725 \pm 169 a	182 \pm 16 a	151 \pm 15 bc	116 \pm 6 a

Gm H1. This finding doesn't correspond with the conclusion of the other authors, for example Liu *et al.* (2002) said that AMF can improve the uptake of K, Ca, and Mg from soil.

CONCLUSIONS

The yield of the tomatoes was influenced by the basic dose of the fertilization, especially in the H2 and H3 variant. The highest yield was in the H2 variant in the variant Gm (938g per plant) in comparison to Ctrl (838g per plant), which makes

the increase of 10%. In the H3 variant, the highest yield was in the variant Gm (913g per plant) in comparison to the control (782g per plant), which is an increase of 14%. The positive effect of the inoculation resulted in the increased content of the vitamin C. The most significant influence was observed in the H2 variant (plants inoculated with Gi) with the average content of the vitamin C 289 mg.kg⁻¹. The increase in the comparison to the control (230 mg.kg⁻¹) represented 20%. The average rate of the colonization was in the range from 39% to 65%.

SUMMARY

The pot experiment was located outdoors under the field conditions at the Faculty of Horticulture in Lednice. The experiment contained 54 containers with tomato plants under three different fertilization regimes (optimum – H1, stress a – H2, stress b – H3) and three different treatments (control – Ctrl, *G. mosseae* BEG95 – Gm, *Glomus intraradices* BEG140 – Gi). 10g, 5g and 2.5g respectively of calcium sulphate (14 % sulfur content) were added in H1, H2 and H3 fertilization treatment respectively. 10g, 5g and 2.5g respectively of bone meal (calcium phosphate content of 80%) were added in H1, H2 and H3 fertilization treatment respectively.

Economical parameter (yield), nutritional characteristic (vitamin C content, phosphates and minerals content, total antioxidant capacity), and level of root colonization were studied. The results of this study indicate that level of P fertilization can influence the level of the mycorrhizal colonization. The colonization was higher with a lower level of P fertilization. The yield of tomato was positively influenced by the mycorrhizal treatment mainly at a lower level of the fertilization. The content of vitamin C was influenced mainly with the mycorrhizal treatment, notably at a lower level of the fertilization. The positive effect of inoculation on the antioxidant capacity was mainly found in the treatment with the highest dose of the fertilization. At lower level of fertilization, the highest content of TAC was found in control plants. The content of PO₄ was positively influenced by the mycorrhizal treatment only in the highest level of the fertilization. In another level of fertilization, no differences were found between treatments, the highest content of PO₄ was in accordance to the highest level of the fertilization as well. The analysis of SO₄ showed the positive effect of the mycorrhiza, mainly in a higher level of the fertilization. In the analysis of K, Na, Mg, no significant differences were found.

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