Comparison of lycopene, β-carotene and phenolic contents of tomato using conventional and ecological horticultural practices, and arbuscular mycorrhizal fungi (AMF)

Comparación del contenido de licopeno, β-caroteno y fenoles en tomate aplicando un manejo hortícola convencional y ecológico y hongos formadores de micorrizas arbusculares (HFMA)

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ABSTRACT

Tomato fruits are rich in anti-oxidant compounds that have been recognized as beneficial for human health. Horticultural practices can influence the concentration of these secondary metabolites. Arbuscular mycorrhizal fungi (AMF) can increase nutrient and water absorption of plants. The experiment, performed under glasshouse, examined whether organically grown 'Vitella F1' tomatoes differed in their fruit content of lycopene, β-carotene and total phenols from that found in conventionally grown tomatoes. Treatments were the cultivation methods: conventional, organic, conventional+AMF and organic+AMF. When comparing the cultivation method, no significant differences for the analyzed nutritional parameters were found; only tomatoes grown organically had slightly lower total phenolic contents. In both cultivation methods, tomato plants inoculated with AMF (*Glomus sp.*) built higher lycopene content in fruits than those without inoculation. Organic grown tomatoes increased β-carotene and total phenolic contents in fruits as a result of the AMF treatment. AMF applications increased root fresh weight but not shoot fresh weight. The improved growth and nutrient acquisition in tomato demonstrated the potential of AMF colonization for increased antioxidant compounds in fruits. White fly (*Trialeurodes vaporariorum*) was controlled successfully with application of diatomaceous earth Fossil Shield® 90 in organic treatments as well as with Applaud® in the conventional cultivation methods.

Key words: ecological farming, mycorrhiza, carotenoids, antioxidants, nutraceutic properties, white fly, diatomaceous earth.

RESUMEN

Los frutos de tomate son ricos en compuestos antioxidantes que han sido reconocidos como benéficos para la salud humana y los manejos hortícolas pueden influir la concentración de estos metabolitos secundarios. Los hongos formadores de micorrizas arbusculares (HFMA) pueden influir en la absorción de nutrientes y agua por la planta. El estudio, llevado a cabo en invernadero, examinó si tomates 'Vitella F1', cultivados orgánicamente, se diferencian en su contenido de licopeno, β-caroteno y fenoles totales en los frutos de tomates manejados convencionalmente. Los tratamientos fueron los métodos de cultivo: convencional, orgánico, convencional+HFMA y orgánico+HFMA. Comparando los métodos de cultivo, no se encontró ninguna diferencia significativa para los parámetros nutritivos analizados; solamente tomates manejados orgánicamente tuvieron un contenido de fenoles totales un poco menor. En los dos métodos de cultivo, las plantas inoculadas con HFMA (*Glomus sp.*) formaron contenidos más altos de licopeno en los frutos que los sin inoculación. Los tomates manejados orgánicamente aumentaron su concentración de β-caroteno y de los fenoles totales a consecuencia del tratamiento con HFMA. La aplicación de HFMA aumentó el peso fresco del fruto pero no el del tallo. El aumento del crecimiento y la adquisición de los nutrientes en tomate demostraron el potencial de la colonización con HFMA para incrementar la concentración de compuestos antioxidantes en el fruto. La mosca blanca (*Trialeurodes vaporariorum*) fue controlada exitosamente con la aplicación de la tierra diatomaceous Fossil Shield® 90 en los tratamientos orgánicos y con Applaud® en el cultivo convencional.

Palabras clave: cultivo orgánico, micorriza, carotenoides, propiedades nutracéuticas, mosca blanca, tierra diatomaceous.

Introduction

Tomatoes (*Lycopersicon esculentum* Mill.) are one of the most popular vegetables products. Worldwide 124,000,000 t of tomatoes were consumed in 2005 (PHN, 2007). For example, the annual *per capita* consumption in Germany is about 19 kg, of which about 7 kg are fresh tomatoes and 12 kg tomato products (Pauli, 2006). In Colombia, tomatoes are the second most important vegetable crop with a production area of about 8,688 ha (241.987 t) in 2006

Fecha de recepción: septiembre 18 de 2007. Aceptado para publicación: abril 9 de 2008

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and a per capita consumption of 9.34 $kg \cdot a^{-1}$ (PNH, 2007). Tomatoes are rich in nutrients, especially potassium, folic acid, and vitamin C, and contain a mixture of different carotenoids, including vitamin A effective ß-carotene, as well as lycopene (Wilcox *et al*., 2003). Variation in the concentration of these compounds has been the focus of much recent research on how genetics and cultural management contribute to fruit composition (Dumas *et al.,* 2006). Fruits and vegetables have received particular attention from researchers and nutritionists because they contain high amounts of known antioxidants such as polyphenols, ascorbic acid, tocopherol, β-carotene, lycopene and many others (Lenucci *et al*., 2006).

Lycopene, in contrast to other carotenoids which are provable in a high number of different fruits and vegetables, is found mainly in few red varieties of fruits and vegetables, in which it is responsible for their typical color. High concentrations of lycopene contain tomatoes and tomato products, but also water melons and pink grapefruits, as well as carrots. Ripe tomatoes especially have high lycopene content. The average lycopene supply for human consumption in Germany is 1.28 $mg \cdot day^{-1}$, of which tomatoes and especially tomato products provide the most important sources of lycopene (Pelz *et al.,* 1998). In contrast to many other secondary plant compounds, the bioavailability of lycopene does not decrease automatically by processing, but rather can be significantly increased (Gartner *et al*., 1997).

Carotenoids, which include lycopene and β-carotene, are found in species that tend to be the most effective naturally existing radical sequesters for oxygen (DiMascio *et al*., 1989). Oxygen radicals are formed, for example, by photo-chemical reactions during light absorption and are highly reactive. They can oxidize different amino acids in proteins, nucleic acids and unsaturated fatty acids. Franceschi *et al.* (1994) found in a statistical investigation, in the North of Italy, that the consumption of tomatoes rich in lycopene leads directly to a decreased incidence of cancer in mouth, pharynx, esophagus, stomach, large intestine, and rectum. In numerous epidemiological studies, a positive correlation was also found between uptake of carotenoid and lycopene and lower cancer diseases and less cardiovascular diseases (Levy *et al*., 1995; Khachik *et al*., 1995; Nagasawa *et al*., 1995; Park *et al*., 1998; Jain *et al*., 1999; Lavelli *et al*., 2000).

For some years now ecological culture practices have increased in many countries. With ecological farming, a holistic thought stands in the foreground which connects social, ecological and economic aspects of the long-term soil - plant animal - human being relationship. Nowadays many discussions center around the extent to which ecological cultivation procedures affect value-giving secondary plant contents (Brandt and Molgaard, 2001).

Myccorhizas are the most widespread associations between microorganism and higher plants (Marschner, 2002). Arbuscular mycorrhizal fungi (AMF) belong to the order Glomales (Zygomycota), are obligatorily biotrophic and form mutual symbiosis with about 80% of field-grown plants (Barea and Jeffries, 1995, Öpik and Rolfe, 2005). The fine roots that perform most of the uptake process are symbiotically associated with fungi which improve nutrient uptake, drought and frost tolerance and protect higher plants against pathogens. The fungi withdraw glucose form plant roots and act as a significant sink for carbohydrates (Kottke, 2002). As a rule the fungus is strongly or wholly dependent on the higher plant, whereas the plant may or may not benefit (Marschner, 2002).

The present work aims to examine how conventional and ecological cultivation methods, and the application of arbuscular mycorrhizal fungi, affect the powerful antioxidant compounds lycopene, β-carotene and total phenols in greenhouse grown tomatoes.

Materials and methods

Tomato culture and application of mycorrhiza

The following signs represent ecological tomato cultivation in contrast to conventional cultivation: seedlings only from ecological plant production, creation of optimum crop conditions and robust plants, longer distances between plants for better aeration, production grown only in the ground and an exclusively use of organic fertilizers.

The foliar and brown rot-tolerant tomato variety Vitella F1 (Fa. Nebelung, Kiepenkerl Pflanzenzüchtung) was used. In Berlin, Germany, seedlings were sown in a greenhouse at the end of April. Weeds were removed manually during the whole experiment period. Tomatoes were cultivated in pairs of of 5 m length rows (experiment group), with three repetitions and 2.5 plants/m. The experiment groups were randomized in three groups with an empty space of 2 m between groups.

The plants were bound once per week or two weeks on an aluminum stick according to length of growth. Water was supplied automatically through drip irrigation. In the conventionally grown tomatoes, a complete fertilizer (N:P:

K:Mg 15:5:20:2) was used applying 50 g·m^{-2} of this fertilizer to the crop and after 60 days a second application of the same amount. The ecological grown tomato plants were fertilized with 3 L compost/m² at the same time. Harvest occurred 10-13 weeks after plantation.

The content of plant-available nitrogen was not measured with the standard soil analysis because it could change rapidly and these compounds are barely stored in soil, instead they leached quite quickly. Due to the high N demand of tomato crops during their vegetation cycle, they had to be post-fertilized at the beginning of the experiment.

For the mycorrhizal trials, tomato seeds were sterilized and were sown in sterile Vermiculite (particles of 3-8 mm; Fa. Kakteen Schwarz) for germination. The seedlings were transplanted in 1.5 L pots in 1:1 sand:vermiculite mixture. They were then inoculated with an arbuscular mycorrhizal fungus (AMF) (*Glomus sp.* spores on expanded clay, Fa. Amykor) and transplanted into sterilized substrate in a greenhouse incorporating 10 g expanded clay near the roots of each plant. The control plants received the same amount of expanded clay, however without inoculum. The plants were kept at 25/18°C day/night temperatures and 70% relative humidity.

To examine the effects of mycorrhization, five plants were harvested for every cultivation method with five repetitions per plant, six weeks after transplanting in the greenhouse, and root weights were obtained. The AMF was evaluated by staining 1 g of root mass, according to Phillips and Hayman (1970), and evaluated as described by Giovannetti and Mosse (1980).

Plant protection

In the conventionally cultivated tomatoes, control of the white fly *Trialeurodes vaporariorum* Westwood was performed through the application of Applaud® (tab. 1). In the ecological grown tomatoes, diatomaceous earth (DE) Fossil Shield® 90.0s (1 g·m^{-2} leaf surface) was applied electrostatically 61 days after sowing. Because many pests stay on the undersides of the leaves the diatomaceous compound was applied electrostatically. This method employs electrostatic forces of attraction that place a surface charge on the spray particles which increases spray deposition on plant surfaces (Law, 2001). Through this, a steady covering of the leaf upper and underside was achieved. One day after application of DE, dust was washed from the leaves by the greenhouse sprinkler system to minimize reduction of photosynthetic rate (further details can be found at Ulrich *et al.*, 2008). After that, nettle broth was applied weekly with a hand spray bottle.

The big nettle (*Urtica dioica* L.) contains high amounts of iron and other mineral substances, flavonoids, carotenoids, vitamins A and C, phosphorus, potassium, nitrogen (of it 40% of ammonium nitrogen which is quickly plant-available) and, most importantly, silicic acid which improves cell wall defenses. For the production of the nettle broth 1 kg of fresh nettle herb was cut in small pieces in 10 L of water, was brought for 5 min to cooking and 24 h allowed to draw. Before applying, broth was cleaned by a sieve.

The monitoring of the efficacy of pest control methods occurred through the application of yellow plastic boards. These were hung throughout the crop during the whole experiment period at a distance of 1 m and the number of caught adult *T. vaporariorum* evaluated weekly.

Soil analysis

Before transplanting of seedlings, three soil samples from the upper 20 cm were taken in three repetitions for every cultivation method (tab. 2). These soil samples were mixed and analyzed for every repetition separately according to standard procedures for nutrients, conductivity and pH-value.

Fruit analyses

Tomatoes were harvested 110 days after planting. For every repetition, 10 ripe tomatoes were harvested at random. The tomatoes were cut in small pieces immediately after

TABLE 1. Plant protection methods used in tomato crops.

Culture system	Problem	Protection strategy
Conventional	White fly, Leaf aphids	Yellow boards, Applaud® (250 g·L^{-1} buprofezin) One application at 0.27 L·ha^{-1}, 61 days after sowing with the first incidence of white fly.
	Grey mold (*Botrytis cinerea*)	Teldor (500 g·kg^{-1} fenhexamid) once, 68 days after sowing.
Ecological	White fly, Leaf aphids	Yellow boards, additional weekly applications with nettle broth, starting from eight weeks after sowing.
		61 days after sowing electrostatic application of diatomaceous earth Fossil Shield® 90.0s.

TABLE 2. Soil analysis before the start of the experiment.

Culture	pH-value	E.C. (dS·m^{-1})	Total N (%)	P (ppm)	K (ppm)
Conventional	7.4 ± 0.4	0.30 ± 0.2	0.164 ± 0.06	51.6 ± 5.2	88.0 ± 8.0
Ecological	6.8 ± 0.3	0.25 ± 0.2	0.150 ± 0.05	75.5 ± 4.9	74.5 ± 6.4

harvest, homogenized and filtered. The filtrate was kept at -70°C for the further analysis.

To analyze carotenoids, 15 g of the filtrate were homogenized in 150 mL hexane acetone mixture (6:4) and afterwards supplied with 0.1 g $MgCO_3$. Then the acetone was removed by washing fivetimes with a saturated NaCl solution. The rest of the hexane solution was filtered at 0.45 µm and analyzed by high pressure liquid chromatography (HPLC). The solvent was an acetonitril-methanol mixture (3:1), the HPLC equipment was from Fa. Dionex.

The total phenol contents were determined with Folin-Ciocalteau reagent (Fa. Merck). In the very alkaline environment, the total polyphenol contents are transformed by Folin-Ciocalteau reagent into a blue colour, whose concentration could be measured photo-metrically and calculated using straight line calibration as described by Singleton and Rossi (1988).

Statistical evaluations

The mean comparison was performed for the fruit content analyses according to a variance analysis of Tukeys HSD-test with a confidence level of 5%.

Results

Six weeks after myccorhizal inoculation no differences were found between the control and the AMF application in shoot weight; however, significant differences in the root mass were found (tab. 3). An AMF colonization of the roots could be observed under light microscope, where intercellular hyphae as well as arbuscules predominated.

Between organically and conventionally cultivated tomatoes, no difference in lycopene and β-carotene contents were found, but total phenolic contents were lower when ecological culture practices were used (tab. 4). On the contrary, AMF application could increase lycopene contents of tomato fruits significantly under conventional and ecological cultivation methods, while β-carotene content was increased by AMF significantly in ecologically cultivated tomatoes compared to those without mycorrhizal inoculum. Total phenolics increased in ecological cultivated tomates with AMF application (tab. 4).

TABLE 3. Shoot and root fresh weight of tomatoes with and without mycorrhiza treatment, harvested six weeks after planting.

	Control	AMF-application
Shoot weight (g)	22.40 a*	23.52 a
Root weight (g)	7.08 a	8.57 b

*Means between the two culture systems (same line) followed by different letters are significantly different at $P \leq 0.05$ using Tukey's HSD-Test.

Starting from the second cultivation week, adult *T. vaporariorum* were found on the yellow traps (fig. 1). The population increased in both cultivation treatments up to the 8th week. The application of Applaud® or Fossil Shield® 90.0s reduced *T. vaporariorum* population clearly. In the conventional cultivation, after the Applaud® application in the 8th week, a slow population increase up to week 13 was observed. Compared to this curve, the population increase after the DE treatment was lower in the ecological cultivation due to the weekly application of nettle broth.

Beside the white fly other pests, such as green peach aphid (*Myzus persicae*) and black bean aphid (*Aphis fabae*), were still found (in low population strength).

Discussion

The higher root mass of AMF plants (tab. 3) is in agreement with Taiz and Zeiger (2006) that root weight increases with the application of fungal material, which is unlikely to exceed 10% of the root weight. Additionally, the better exploitation of the soil for inorganic nutrients and water

TABLE 4. Fruit substances related to fresh weight for conventionally and ecologically cultivated tomatoes, with and without mycorrhiza treatment.

Cultivation method	Lycopene (ppm)	β-carotene (ppm)	Total phenolic content (ppm)
Conventional	9.87 a*	0.058 a	8.5 b
Ecological	9.37 a	0.061 a	7.9 a
Conventional + AMF	10.55 b	0.083 ab	8.3 b
Ecological + AMF	11.00 b	0.090 b	8.6 b

*Means of plant substances between the different cropping systems (same column) followed by different letters are significantly different at $P \leq 0.05$ using Tukey's HSD-Test.

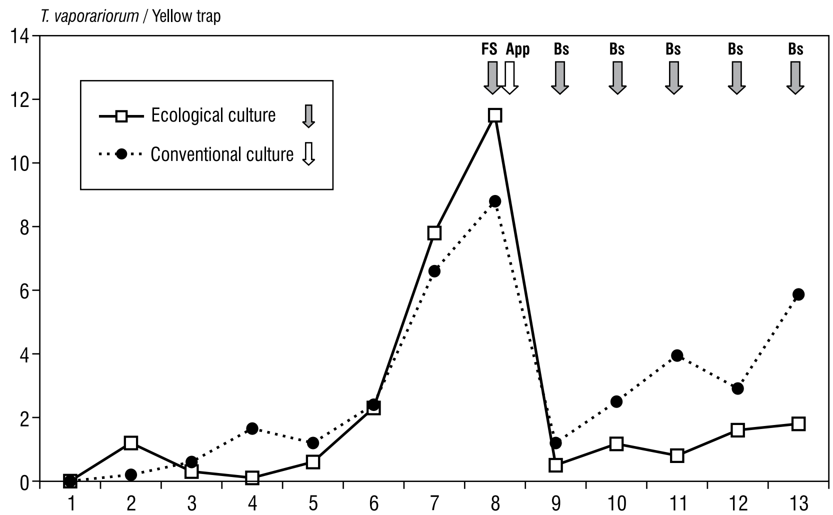

FIGURE 1. Development of the white fly *Trialeurodes vaporariorum* over the whole experiment time in conventionally and ecologically cultivated tomatoes as well as plant protection with insecticides. Application of FS = Fossil Shield 90.0s; App.= Applaud (Buprofezin); Bs = Nettle broth.

(Schopfer and Brennicke, 2006) and the higher transport rates of assimilates from the aerial plant parts towards the roots (Mengel and Kirkby, 2001) in mycorrhizal infected plants could have played a role in enhancing root weight of these treatments. Also Manjarrez-Martínez *et al.* (1999) found higher root volume in chili plants inoculated with *Glomus spp.* than in those without (control) or with vermicompost, which they considered as caused by a direct effect of the mycorrhiza whereas increased assimilation of nutrients could not be exclusively attributed to the mycorrhizal hyphae but also to enhanced root growth of the host (Marschner and Dell, 1994), resulting in a higher export of nutrients and water to the aerial part. Mycorrhized tomato plants did not produce higher shoot mass, contrary to findings of Al-Karaki (2000).

The proved lycopene content amounted only approximately one third those found by Holdon *et al.* (1999) for tomato fruits; however, it is known that the content can strongly vary according to the variety (López *et al.,* 2001, Toor *et al.*, 2005). In general, the study showed that powerful antioxidant compounds such as lycopene and β-carotene were not reduced by organic culture practices. Although limited to only a few rigorous controlled studies (Lester, 2006), the literature supports the popular belief that organic fertilizer is superior to conventional synthetic fertilization in achieving more nutritious fruits or vegetables (Asami *et al.*, 2003). This hypothesis could not be confirmed in this experiment, especially since total phenolic content decreased in the ecological cultivated tomatoes. Nor was it confirmed that the lower nitrogen concentration in the ecological cultivated soil is correlated positively with the production of secondary plant metabolites, such as phenols (Norbaek *et al.*, 2003).

Since Fanasca *et al.* (2006) described that a high proportion of K in the nutrient solution increased quality attributes such as lycopene content, this may have played a role in our experiment because ecological soil had lower K content than the conventionally one and this could have led to the fact that the ecological cultivated tomatoes did not increase lycopene content. Also no differences were found between conventionally and ecologically cultivated tomatoes in β-carotene content, which are supposed to be observed because lycopene is one of the precursors of β-carotene synthesis formed through cyclization of lycopene (Heldt, 2003).

In general, ecological treatments with AMF-application increased carotenoid and total phenolic contents, while myccorhizal inoculation also increased lycopene content in conventionally cultivated tomatoes. A stimulation of the carotenoid metabolism in arbuscular mycorrhizal roots was already described by Fester *et al.* (2002). The enhancement of water and soil nutrient uptake through AMF inoculation can enhance photosynthetic performance (Schopfer and Brennicke, 2006), which in turn triggers an increase in synthesis of carotenoid pigments. Carotenoids, which serve as accessory pigments in photosynthesis and also as photoprotective agents, are isoprenoid (terpenoid) compounds, which originate in the primary carbon metabolism (Taiz and Zeiger, 2006).

The successful control of white fly (*Trialeurodes vaporariorum)* with diatomaceous earth Fossil Shield® 90 in organic treatments was the result of insect dehydration. This dehydration is a response to damage to the protective wax layer of the arthropods, in which cuticle lipids are absorbed by the silicate-containing particles and sink even into the wax layer of the insects (Mewis and Reichmuth, 1999; Mewis and Ulrichs, 2001b; Prashanta, 2003).

Conclusions

- Growing tomatoes with ecological crop methods not only can protect air, plant and soil from contamination, but also maintain fruit antioxidant compounds such as lycopene and β-carotene. Only phenolic content decreased slightly as compared to conventionally managed plants.

- Tomato plants inoculated with AMF (*Glomus sp.*) built higher lycopene and β-carotene content in fruits than those without inoculation, indicating that the higher nutrient and water uptake of mycchorizal plants increase growth and photosynthesis and in turn carotenoid synthesis.

- Electrostatic applied diatomaceous earth Fossil Shield® 90 can control white fly (*Trialeurodes vaporariorum)* successfully in ecological cultivation methods.

Acknowledgements

The authors wish to thank to Dr. Rebecca Lee and Dr. Stanislav Magnitskiy for critical reading of the English text.

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