Scientia Horticulturae 144 (2012) 55–59

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Yield and nutrient content of tomato (*Solanum lycopersicum* L.) as influenced by *Trichoderma harzianum* and *Glomus mosseae* inoculation

Bombiti Nzanza[a,*], Diana Marais[a], Puffy Soundy[b]

[a] Department of Plant Production and Soil Science, Faculty of Natural and Agricultural Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa
[b] Department of Crop Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

ARTICLE INFO

Article history:
Received 2 June 2011
Received in revised form 10 April 2012
Accepted 5 June 2012

Keywords:
Mycorrhizae
Nutrient uptake
Phytochemical content
Solanum lycopersicum
Trichoderma harzianum

ABSTRACT

Recent trends in soil microbiology suggest that fungal inoculants such as *Trichoderma harzianum* or arbuscular mycorrhizal fungi (AMF) have the potential to improve yield and fruit quality of crops. The purpose of this study was to investigate the effect of inoculating tomato (*Solanum lycopersicum* L.) with *T. harzianum* and the AMF (*Glomus mosseae*) on yield and nutrient content of tomato fruit. A factorial experiment (3 × 3) with three application timings for each of *T. harzianum* and AMF, namely uninoculated control, inoculated before sowing and two weeks after sowing, giving nine treatment combinations was conducted in a greenhouse. Both *T. harzianum* and AMF increased total yield and marketable yield of tomato ($P > 0.05$). Inoculating tomato with AMF before sowing significantly increased the percentage of extra-large fruit, while inoculation with *T. harzianum* two weeks after sowing lowered the Ca and Mg contents of tomato fruit. *T. harzianum* and AMF inoculation increased the lycopene content, but did not affect the antioxidant activity, total flavonoids or vitamin C of the tomato fruit. Results of this study suggested that *T. harzianum* and AMF have the potential to influence yield and nutrient content of tomato in a greenhouse.

1. Introduction

Tomato is the second-most important vegetable in the world after potato (Dorais et al., 2008), with a worldwide production of 129 million tons in 2008 (FAO, 2010). It is an excellent source of health-promoting compounds due to the balanced mixture of antioxidants including vitamins C and E, lycopene, beta-carotene, lutein and flavonoids (Dorais et al., 2008), amino acids, proteins, fatty acids and carbohydrates (Hauffman and Bruce, 2002; Heeb, 2005). Tomato is also rich in macronutrients, especially K (Wilcox et al., 2003; Odriozola-Serrano et al., 2009), P, Mg and Ca (Súarez et al., 2008) and contains high amounts of trace elements such as Fe, Mn, Zn, and Cu (Ahmed et al., 2011). Nutritional studies have suggested that regular consumption of fruits and vegetables, including tomatoes, can play an important role in preventing cancer and cardiovascular diseases in humans (Heber, 2000; Rao and Agarwal, 2000; Toor and Savage, 2005).

Since tomato fruit play an important role in human health (Chapagain and Wiesman, 2004), strategies for increasing fruit production and quality are of great interest to producers (Gruda, 2005; Flores et al., 2010). Compelling evidence in literature suggest that mineral nutrients can affect the antioxidant content of tomato fruit and overall tomato fruit quality. For instance, increased Ca levels in soil solution increase Ca content in tomato fruit, but decrease carotene content and lycopene levels (Paiva et al., 1998). Adequate Ca supply is essential for fruit firmness and extended shelf life (Cooper and Bangerth, 1976). Increasing K increases carotenoid concentration, particularly the lycopenes (Trudel and Ozbun, 1971). According to Mozafar (1994), beta-carotene content in fruit increases with increasing levels of K, Mg, Mn, B, Cu and Zn, whereas Lester (2006) reported that ascorbic acid increased with increasing levels of K, Mn, B, Cu and Zn. Phosphorus may also increase the fruit concentration of phytochemicals such as ascorbic acid, flavonoids and lycopene (Dorais et al., 2008). The need for producing of high quality food, while mitigating deleterious environmental impact (Mader et al., 2002) makes the use of biofertilisers a preferred alternative and feasible production practice in contrast to the use of inorganic fertilisers (Mena-Violante and Olalde-Portugal, 2007).

Indications are that *Trichoderma harzianum* can improve the solubility of soil micronutrients, such as Zn, Cu, Fe, Mn (Kaya et al., 2009) whereas arbuscular mycorrhizal fungi (AMF) enhance the uptake of P, N and K (Cardoso and Kuyper, 2006). However, information regarding their combined effects on the phytochemical content, nutrient content and yield of tomato is inconsistent (Gosling et al., 2006), inadequate (Dumas et al., 2003) or simply lacking. The objective of this study was to determine the effect of tomato root inoculation with *T. harzianum* and AMF on fruit yield,

* Corresponding author. Tel.: +27 83 627 4861; fax: +27 15 395 2106.
E-mail addresses: s24493024@tuks.co.za, bombiti@zz2.biz (B. Nzanza).

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http://dx.doi.org/10.1016/j.scienta.2012.06.005

fruit quality and nutrient content of tomato fruit produced in a greenhouse system.

2. Materials and methods

2.1. Study location, microbial inoculants and seedling production

This study was conducted under greenhouse conditions at the Hatfield Experimental Farm, University of Pretoria, South Africa during the 2009 growing season. The site is located at 23°45′S latitude, 28°16′E longitude, at a 1372 m above sea level. Seeds of tomato cv. 'Nemo-Netta' were sown into cell plug trays filled with peatmoss, thoroughly mixed with the appropriate treatment and covered with vermiculite.

Commercial mycorrhizal inoculum Biocult© (Sommerset West, South Africa), containing spores of *Glomus mosseae* (40 spores g^{-1}, as granulate) was obtained from Biocult Ltd. (Sommerset, South Africa), whereas commercial *T. harzianum* inoculum containing spores of *T. harzianum* isolate DB 103 (1×10^9 CFU g^{-1}, as a wettable powder) was obtained from Dagutat Biolab (Cape Town, South Africa).

Seedling trays were divided into three groups, with the first group being inoculated with AMF that was thoroughly mixed with peatmoss at the rate of 10 g kg^{-1} peat before sowing. In the second group (two weeks later), AMF was applied in seedling trays before transplanting around the basal part of the plant. The last group, comprising untreated AMF, had the same amount of autoclaved inoculum applied. In addition, the non-AMF control received a 10 mL aliquot of AMF filtrate to establish similar microflaura communities. Each seedling tray group was later divided into three groups corresponding to *T. harzianum* application, which were (1) applied at sowing, (2) two weeks later or (3) untreated control. The inoculum was added to reach a population of 1.8×10^7 conidia g^{-1} peat.

2.2. Experimental design and treatments

Two weeks after sowing, seedlings were transplanted to 5 L-pot filled with an autoclaved sand coir mixture (ratio 2:1). The nine treatment combinations (3 *T. harzianum* × 3 AMF), T_0M_0 (uninoculated), T_0M_1 (treated with AMF only, before sowing), T_0M_2 (treated with AMF only, two weeks after sowing), T_1M_0 (treated with *T. harzianum* only, before sowing), T_1M_1 (treated with both fungi before sowing), T_1M_2 (treated with *T. harzianum* before and AMF two weeks after sowing), T_2M_0 (treated with *T. harzianum* only, two weeks after sowing), T_2M_1 (treated with *T. harzianum* at two weeks after sowing and AMF before sowing) and T_2M_2 (treated with both fungi two weeks after sowing), were arranged in a completely randomised design with six replications. Plant pots were spaced at 0.4 m between plants in a double row with 1 m between rows. Plants were fertilised with half strength modified Hoagland's solution (Spomer et al., 1997) and watered daily.

2.3. Data collection

2.3.1. Harvest

Harvesting started at 10 weeks after transplanting and was continued for ten successive weeks, with two harvests per week. Twenty fruit/replicate of colour stage six, using tomato colour chart standard (Kleur-stadia tomaten, Holland), were used for fruit quality analysis. Fruit were divided into two groups as representative samples for the two fruit quality analysis procedures with the first group being used for the determination of the macro-elements, whereas the second group was used for the analysis of antioxidant activity, vitamin C, lycopene and total flavonol contents.

2.3.2. Yield and yield components

At each harvest, fruit of colour stage six were weighed for total yield determination. The marketable yield was calculated as the weight of the total number of the fruits per plant (total yield) minus the weight of small fruits (<47 mm) and unmarketable fruits (defects, disease or physiological disorders). Fruit diameter was measured with a digital caliper (Starreett, 727 Series, Athol, MA, USA) and divided into four categories, using Jones (1999) scale: extra-large (>67 mm), large (54–67 mm), medium (47–54 mm) and small (<47 mm).

2.3.3. Fruit quality

Phytochemical content in fruit analysis was performed at Limpopo Agro-food Technology Station, Polokwane, South Africa. Lycopene content was extracted from tomatoes with a hexane–acetone–ethanol (2:1:1) mixture using Sharma and Le Maguer's (1996) and Toor's et al. (2006) methods. Vitamin C content was measured by a Metrohm 670 titroprocessor (Metrohm Herisau, Switzerland) using the method of the Association of Official Analytical Chemists (AOAC, 1990; Toor et al., 2006). Antioxidant activity was estimated by the Trolox Equivalent Antioxidant Activity method (Miller and Rice-Evans, 1997). Flavonoid content was measured using a colorimetric assay (Zhishen et al., 1999). Total P, K, Ca and Mg were determined by microwave digestion followed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (USEPA, 1986).

2.4. Data analysis

Data were subjected to two-way analysis of variance using SAS (SAS Institute Inc., Cary, NC, USA) (2002–2003) with *T. harzianum* and AMF as treatments factors, each at three levels (untreated, at sowing, and two weeks later). The variance was related to the main effects and interactions between them. When *F*-ratio was significant, mean separation was achieved using Fisher's least significant difference test. Unless otherwise stated, treatments discussed were significantly different at 5% level of probability.

3. Results

3.1. Yield and yield components

Main treatments and their interaction had no significant effect on the number of fruit, marketable yield and total yield of tomato per plant (Table 1). Both fungal inoculants increased the yield and marketable yield of tomato as compared to the untreated plants ($P > 0.05$). Mean comparison showed that the highest total yield (8.2 kg $plant^{-1}$) and marketable yield (79.8%) were achieved with the combined inoculation of *T. harzianum* and AMF before seeding (Table 1).

Regardless of *T. harzianum* application, inoculating with AMF before sowing (M_1) increased the percentage of extra-large fruit by about 8% as compared to the uninoculated plants (M_0), which were similar to those inoculated with AMF two weeks after sowing (M_2). In terms of medium fruit, inoculating AMF before (M_1) or two weeks after sowing (M_2) decreased the percentage of fruit by about 23.6% and 15.5%, respectively, when compared with uninoculated plants (M_0) (Table 2).

3.2. Tomato fruit mineral content

There was a significant effect of *T. harzianum* inoculation on Ca and Mg fruit contents (Table 3). Inoculating *T. harzianum* two weeks after sowing (T_2) decreased the fruit content of Ca by about 21% as compared to the uninoculated plants (T_0). Compared to the untreated plants, Mg content of fruit was significantly lowered