Response of tomato (*Solanum lycopersicum* L.) to nursery inoculation with *Trichoderma harzianum* and arbuscular mycorrhizal fungi under field conditions

Bombiti Nzanza*, Diana Marais and Puffy Soundy

Department of Plant Production and Soil science, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0001, South Africa

*Corresponding author. Email: s24493024@tuks.co.za.

Tel: +27153952135. Fax: +27153952135

Abstract

The effect of nursery inoculation of tomato (*Solanum lycopersicum* L.) with *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* on fungal root colonization, plant growth, yield and quality of field grown tomato was investigated. The four treatments included *T. harzianum*, AMF, *T. harzianum* + AMF, and uninoculated control. At mid-harvest, 84 days after transplanting, no interactive effect of the fungi on the external mycelium growth was observed. Inoculation with AMF alone or in combination with *T. harzianum* increased dry shoot weight by 35% and 30%, respectively, during the first season, and by 30% and 21%, respectively, during the second growing season. *Trichoderma harzianum* increased the percentage of large fruit by 76% in 2008–2009, whereas AMF increased the percentage of extra-large fruit by 44% in 2009–2010. Similarly, AMF increased total soluble solids by 10%. Inoculated tomato seedlings with *T. harzianum* and/or AMF significantly increased early yield of tomato, by 10%, 65% and 70%, respectively, during 2008–2009, and by

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27%, 36% and 37%, respectively during the 2009–2010 growing season. In conclusion, results of the study suggested that *T. harzianum* and AMF have the potential to improve growth, early yield and fruit quality of field-grown tomato.

Keywords: *Mycorrhiza*, nursery inoculation, *Solanum lycopersicum*, *Trichoderma*.

Introduction

Conventional tomato growers heavily rely on synthetic fertilizers and pesticides to achieve desirable fruit yield, resulting in soil fertility loss, unbalanced nutrition, nutrient leaching and poor soil quality. Increasing concerns over soil degradation and loss of biodiversity have enthused producers to consider alternative low-input agriculture such as organic farming. In South Africa, some growers make use of *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) in the nursery to improve plant growth and to control soilborne pathogens (Taurayi, 2011).

Trichoderma harzianum* is well-studied as a biological control agent, with indisputable results that have demonstrated the influence of *Trichoderma* strains in disease protection (Datnoff et al., 1995; Tsahouridou & Thanassoulopoulos, 2002), particularly in controlling damping-off in tomato production (Lewis & Lumsden, 2001). Also, *Trichoderma* strains improved tomato vegetative growth and development (Chang et al., 1986; Gravel et al., 2007), but with little evidence of increased yield. However, Bal and Altintas (2008) observed a positive result of *T. harzianum* on lettuce yields, but not on yield of tomato in an unheated greenhouse. Most of the cited studies have concentrated on seedling (Chang et al., 1986; Inbar et al., 1994; Tsahouridou & Thanassoulopoulos, 2002) or greenhouse production (Bal & Altintas, 2006;

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Gravel et al., 2007), with little field research. Even so, when field studies were conducted, the focus was on suppression of soil-borne diseases (Datnoff et al., 1995; Coskuntuna & Özer, 2008), with little attention to yield.

Much work have shown the potential of AMF to enhance mineral nutrient uptake (Smith & Read, 1997), particularly P (Marschner & Dell, 1994), alleviation of stresses such as drought (Nelsen & Safir, 1982; Subramanian et al., 2006) and salinity (ZhongQun et al., 2007) and the suppression of soil borne diseases (Hooker et al., 1994). Subramanian et al. (2006) found an improvement in fruit production and drought tolerance of AMF-inoculated tomato plants due to enhanced nutritional status of the plants. Al-Karaki (2006) reported an increase in yield and alleviation of deleterious salt stress following inoculation with AMF. Although Bolan et al. (1984) found an increase in mycorrhizal colonization in subterranean clover with increased P application; the general belief is that AMF performs poorly under optimal soil nutrition conditions (Strzemska, 1975). Due to the symbiotic nature of interaction between AMF and the host, which is based on bidirectional nutrient exchange (Karandashov & Bucher, 2005), it is unclear as to whether under optimum field conditions AMF would benefit the host plant or simply become a parasite. When inoculated simultaneously under greenhouse conditions, *T. harzianum* and AMF had the potential to improve tomato vegetative growth (Nzanza et al., 2011).

The interaction of *T. harzianum* and AMF under field conditions is not well-documented. The objective of this study was to determine the effect of root inoculation with *T. harzianum* and AMF on fungal root colonization, vegetative growth, fruit yield and quality of tomato.

Material and methods

Site description

Field trials with drip irrigated tomatoes were conducted during the November-May growing season of 2008–2009 and repeated in 2009–2010 at Vreedsaam farm, ZZ2-Bertie van Zyl, Mooketsi, South Africa. The site is located at 23º65'17"S latitude, 30º06'89"E longitude, at 772 m above sea level, in the northern part of South Africa. Mean day/night minimum temperatures ranged from 23ºC /15ºC to 21ºC/15ºC, whereas mean day/night maximum temperatures ranged from 28ºC/23ºC to 29ºC/25ºC. A rainfall of 451 mm and 354 mm was received during the respective growing seasons.

Soil sampling and analysis before planting

Soil samples were randomly collected at depth of 0–30 cm using a soil auger (7.5 cm in diameter and 20 cm depth). Composite samples were mixed thoroughly, air-dried and sieved to pass through a 2 mm screen for physico-chemical analysis and mycorrhizal spore counts. Soil pH was determined in a 1:2.5 suspension (soil/water), whereas the Walkley-Black (1934) method was used to determine the total organic carbon. Soil K was determined using the flame photometer, while soil Ca and Mg were determined with an atomic absorption spectrophotometer. Soil available P was extracted with Bray 2 solution and determined with a spectrophotometer. The weight-sieving technique was used for mycorrhizal spore counts (Brundrett et al., 1996), while the hydrometer method was adopted for soil texture analysis (Kalra & Maynard, 1991).

The soil had a pH (H_2O) of 5.9 with 10 mg kg^{-1} P, 202 mg kg^{-1} K, 194 mg kg^{-1} Mg, 731 mg kg^{-1} Ca, and organic carbon of 1.5%. The mycorrhizal spore propagules on the site were less

than one kg^{-1} soil, thus the soil was not fumigated. Soil at the experimental site comprised of sandy loam with 80% sand, 14% clay and 6% silt. The field experimental was divided into two portions having similar soil texture and nutrient status, with the first planted in 2008–2009, whereas the second was used during the 2009–2010 growing season.

Treatments

Treatments consisted of inoculating the growing media with *T. harzianum* alone, AMF alone, or *T. harzianum* + AMF before sowing and the uninoculated control. In the AMF treatments, commercial mycorrhizal inoculum Biocult$^{©}$ (Sommerset West, South Africa) containing spores of *Glomus mosseae* [(Nicol. & Gerd.) Gerd. & Trappe], was applied at a rate of 10 g kg^{-1} of peat, whereas commercial *Trichoderma* inoculum T-GRO (Dagutat Biolab, Johannesburg, South Africa) containing spores of *T. harzianum* isolate DB 103 was added to reach a population of 1.8×10^7 conidia g^{-1} peat. Seeds of tomato 'Nemo-Netta' were sown into cell plug trays filled with peatmoss, thoroughly mixed with the appropriate treatment, covered with vermiculite and allowed to grow for four weeks before transplanting to the open field.

Plant culture

The fields were ploughed and harrowed before constructing 30-cm-high raised beds. Four-week old tomato seedlings were transplanted into double rows on the beds, with a spacing of 30 cm between plants and 180 cm between rows. Each experimental plot measured 20 m in length × 1.8 m in width (36 m^2). Eight weeks before transplanting plots received an organic amendment in the form of compost (10 m^3 ha^{-1}) made from grass clippings, manure, wood chips, sawdust and a mixture of chicken and cattle manure (4 m^3 ha^{-1}) at 1:1 (v/v), which accounted for 50 kg N ha^{-1}, 37 kg P ha^{-1} and 100 kg K ha^{-1}. During both growing seasons, plots received 200 N kg N ha^{-1} as

ammonium sulphate, 23 kg P ha^{-1} as phosphoric acid, 300 kg K ha^{-1} as potassium nitrate, 150 kg Ca ha^{-1} as calcium nitrate and 25 kg Mg ha^{-1} as magnesium sulphate, as side-dressing through drip irrigation. Irrigation was scheduled using evapotranspiration rates of the plants. Standard cultural practices for tomato production were applied. Scouting for pests and diseases with low economical damage was done throughout the trial. Whiteflies and aphids were controlled by drenching the plants with Actara® (thiamethoxam 25%) at label rates of 0.03 ml plant^{-1}. Biomectin® (Abamectin 18 g l^{-1}) was applied at the rate of 0.6 l ha^{-1} for the suppression of leafminer, whereas Kocide® 2000 (copper hydroxide) and mancozeb® 800 WP (dithiocarbamate) were used for suppressing early blight (*Alternaria solani*), bacterial spot (*Xanthomonas vesicatoria*) and bacterial speck (*Pseudomonas tomato*). Weeds were removed by hand pulling or hoeing.

Data collection

Root colonization and dry matter production

Twelve weeks after transplantating, plants were pulled out of the soil, gently washed to remove the soil, and roots were separated from shoots. Roots of tomato plants were stained with trypan blue in lactophenol (Phillips & Hayman, 1970) and quantified for percentage of AMF colonization using the line-intersect method (Brundrett et al., 1996). Root colonization by *T. harzianum* was determined following the procedure described by Datnoff et al. (1995). Root pieces of 1 cm in length, washed and disinfected with 5% NaOCl, were plated on acidified potato dextrose agar or water agar amended with 100 µg streptomycin sulphate. Percentage root infection was determined by counting the number of root pieces containing at least one colony of *T. harzianum* per root segment per plate, then dividing by the total number of root pieces and

multiplying by 10. The remaining roots and shoots were oven-dried at 65ºC for 48h for dry matter determination.

Yield variables

The harvesting period started 12 weeks after transplanting and was carried out for successive weeks, with two harvests per week. At the tenth harvest, 20 fruit/replicate of colour stage 6, using tomato colour chart standard (Kleur-stadia tomaten, Holland), were used for fruit quality analysis. Fruit were analysed for total soluble contents using a digital refractometer, for pH using a pH-meter and dry matter content as described above for dry matter content. At each harvest, fruit were weighed for the determination of total yield, with marketable yield being determined as the total number of fruit per plant (total yield) minus small fruit and unmarketable fruit due to defects, diseases or physiological disorders.

Fruit quality

Individual fruit diameter was recorded with a digital caliper (Starreett, 727 Series, Athol, Massachusetts, USA) and divided into four categories, *viz* extra-large (> 67 mm), large (67–54 mm), medium (54–47 mm) and small (< 47 mm) fruit as described by Jones (1999). The vitamin C content was measured by Metrohm 670 titroprocessor (Metrohm, Herisau, Switzerland) using the method of the Association of Official Analytical Chemists (AOAC, 1990; Toor et al., 2006), fruit juice was extracted and homogenised using a centrifuge for 20 minutes. The supernatant was then measured for total soluble solids (TSS) using a digital refractometer.

Data analysis

Data were subjected to analysis of variance using SAS (SAS Institute Inc., Cary, NC, USA. (2002–2003). Mean separation was achieved using Fisher's least significant difference test. Unless stated otherwise, treatments discussed were different at 5% level of probability.

Results

Mycorrhizal and Trichoderma root colonization

Regardless of the growing season, data showed that non-AMF plants had less than 1% mycorrhizal root colonization, whereas AMF-inoculated plants had a root colonization of above 20% (Table I). For *T. harzianum*, the uninoculated plants had less than 10% root colonization, whereas the inoculated ones had more than 85% root colonization.

Shoot and root weight and ratio

Inoculation with AMF alone or in combination with *T. harzianum* increased dry shoot weight by 35% and 30%, respectively, during the first season, and by 30% and 21% during the second growing season when compared to the uninoculated plants (Table I). On the other hand, the shoot : root ratio was influenced by the fungal inoculation during the 2008–2009 growing season only, with a significant increase due to AMF (35%) followed by *T. harzianum* (32%) when compared to the uninoculated plants (Table I). Combined treatment had no additive effect on shoot : root ratio.

Yield and yield components

The AMF alone or in combination with *T. harzianum* increased early yield of tomato by 70% and 64%, respectively, during the first season, and by 37% and 36% during the second growing

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Table I. Dry shoot: root ratio of field grown tomato as influenced by *T. harzianum* and AMF nursery pre-inoculation (T: *T. harzianum*; M: AMF; TM: combined application of *T. harzianum* and AMF).

Treatment	% AMF colonisation			% *Trichoderma* colonisation			Dry shoot mass g/plant			Dry root mass g/plant			Dry shoot: root ratio		
	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean
M	23.60a	20.00a	21.80	12.00b	5.20b	8.60	30.28a	36.01a	33.15	5.01	5.23	5.12	6.13a	6.97	6.55
T	1.00b	0.80b	0.90	92.00a	79.60a	85.80	30.80a	29.27bc	30.04	5.15	5.43	5.29	6.00a	5.40	5.70
TM	20.40a	22.20a	21.30	94.00a	82.40a	88.20	29.10a	33.47ab	31.29	5.52	5.08	5.30	5.30ab	6.59	5.95
Control	0.80b	1.00b	0.90	4.96b	4.80b	4.88	22.40b	27.61c	25.01	4.96	5.00	4.98	4.53b	5.51	5.02
C.V (%)	44.82	42.87		14.80	39.05		12.07	9.11		8.05	6.63		10.15	9.67	

Means followed by the same letter were not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

Table II. Yield and yield components of field grown tomato as influenced by *T. harzianum* and AMF nursery pre-inoculation (T: *T. harzianum*; M: AMF; TM: combined application of *T. harzianum* and AMF).

Treatment	No. fruit/plant			Marketable fruit/plant			Early yield kg/plant			Total yield/plant kg/plant			Marketable yield kg/plant		
	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean
M	149.40	137.20	143.30	118.20	106.60	112.40	2.79a	2.30a	2.55	8.99	8.68	8.84	7.05	6.62ab	6.84
T	143.20	131.60	137.40	119.40	100.20	109.80	1.80ab	2.14a	1.97	8.38	8.2	8.29	6.23	6.00b	6.12
TM	147.60	141.60	144.60	117.40	112.20	114.80	2.70a	2.28a	2.49	9.02	8.84	8.93	7.18	7.00a	7.09
Control	149.40	138.00	143.70	116.40	102.00	109.20	1.64b	1.68b	1.66	8.07	8.19	8.13	6.03	6.02b	6.03
C.V (%)	7.21	8.59		13.41	10.13		27.77	11.54		9.32	5.39		12.39	12.50	

Means followed by the same letter were not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

season. Fungal inoculants did not increase total fruit yield of tomato (Table II). However, a slight increase (16%) in the marketable yield as compared to the control was obtained with combined inoculation of *T. harzianum* and AMF during the second growing season (Table II). The number of fruit and marketable fruit per plant were not affected by any of the treatments during either season.

Fruit size

Differences among the four treatments with regard to percentage of extra-large fruit during the first growing season were not detected (Table III). However, in the 2009–2010 season, the AMF alone or in combination with *T. harzianum* increased percentage extra-large fruit by 44% and 39%, respectively, while *T. harzianum* increased percentage of large fruit by 76% in 2008–2009 (Table III).

Vitamin C and TSS

All inoculated tomato plants increased vitamin C content of tomato fruit, with only AMF-treated plants recording a significant increase (23%) over the untreated plants in 2008–2009. In 2009–2010 no significant differences were found amongst any of the treatments (Table III). The TSS was increased by 11% and 9%, respectively, under both *T. harzianum* and AMF, each inoculated alone in 2008–2009. However, in 2009–2010 no significant difference was found between *T. harzianum*-inoculated and untreated plants (Table III).

Discussion

Inoculation of tomato seedlings in the nursery with *T. harzianum* and AMF, either alone or in combination, promoted plant growth, fruit quality and early fruit yield of field grown tomatoes.

Table III. Fruit size, TSS and vitamin C content of field grown tomato as influenced by *T. harzianum* and AMF nursery pre-inoculation (T: *T. harzianum*; M: AMF; TM: combined application of *T. harzianum* and AMF).

Treatment	Extra-large fruit %			Large fruit %			Medium fruit %			TSS %Brix			Vitamin C mg/100g FW		
	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean
M	34.82	42.24a	38.53	27.70b	22.88b	25.29	23.84ab	16.00	19.92	5.40a	5.68a	5.54	29.20a	25.10	27.20
T	31.22	35.18ab	33.20	41.18a	32.12ab	36.65	15.22b	16.88	16.05	5.32a	5.62ab	5.47	27.40ab	26.10	25.70
TM	39.10	39.08ab	39.09	30.48b	26.20ab	28.34	16.80b	21.54	19.17	5.28a	5.72a	5.50	26.50b	25.00	26.80
Control	30.30	29.32b	29.81	23.30b	22.88b	23.09	31.58a	25.66	28.62	4.86b	5.26b	5.06	23.80b	22.60	23.20
C.V (%)	23.25	16.27		17.45	15.05		36.41	44.27		7.65	6.47		16.17	14.25	

Means followed by the same letter were not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

Enhanced vegetative growth was not translated into increased yield and yield components of tomato. In fact, most of the increased yield associated with AMF was either due to its potential to alleviate stress such as severe drought (Subramanian et al., 2006), salinity (Kaya et al., 2009) or disease incidence. Kaya et al. (2009) demonstrated that AMF increased fruit yield of salt-stressed tomato plants but not that of non-stressed plants, whereas Al-Karaki (2006) observed higher yields in AMF-inoculated plants than in uninoculated plants. Reports on increased tomato yield with *T. harzianum* are rare, although Gravel et al. (2007) observed an increase in yield with *T. atroviride* in rockwool. Additionally, increased yields in cucumber, bell pepper and strawberry had been reported with *T. harzianum* (Altintas & Bal, 2005; Altintas & Bal, 2008; Bal & Altintas, 2006; Poldma et al., 2002). In this study, although all fungal inoculants induced a negligible increase in yield of tomato, treatment effects were not significant.

Findings of this study also demonstrated the beneficial effect of inoculating seedlings with *T. harzianum* and/or AMF on the earliness of the yield, suggesting that these fungal inoculants have the potential to increase the total yield of tomato. Although data showed that combined inoculation of *T. harzianum* and AMF was more effective than either applied alone, marketable yield increase obtained during the second season was rather due to relatively higher rate of unmarketable yield than the fungal inoculant's effect.

Trichoderma harzianum increased the percentage large fruit in 2008–2009, while AMF increased the percentage extra-large fruit in 2009–2010 growing season. The increased in fruit size by *T. harzianum* and AMF was probably due their ability to trigger enzymes involved in tomato fruit cell expansion. However, combining *T. harzianum* and AMF had little effect on tomato fruit size, when compared to each fungal inoculant alone. These findings are in agreement with Datnoff et al. (1995) who did not find any beneficial effect of dual inoculation of

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tomato with *T. harzianum* and AMF on extra-large fruit. Inoculating tomato seedlings with *T. harzianum* and AMF improved the TSS of tomato fruit. Higher sugar content, obtained with both fungal inoculants, specifically those treated with AMF, suggested that carbohydrate partitioning in the plant was not solely restricted to AMF. However, this finding did not confirm previous observations where a decrease in the fruit TSS was observed in AMF-treated plants in processing cultivars (Martin 2007). The differences in fruit quality parameters between the 2008–2009 and 2009–2010 trials could be attributed to seasonal differences such as rainfall and temperature.

Uninoculated AMF plants had low mycorrhizal colonization (< 1%) due to the low indigenous mycorrhizal count prior to planting, whereas the lower root colonization of AMF-treated plants (about 21%) could be due to chemical input or other variables such as irrigation, timing of fertilizer, or interaction with endemic AMF in the rhizosphere. Chandanie et al. (2009) reported an inhibition of *T. harzianum* around cucumber roots following the application of the AMF *G. mosseae* whereas Calvet et al. (1992) observed a significant enhancement of AMF growth due to the presence of *T. harzianum in vitro*. In this study, *T. harzianum* had no effect on mycorrhizal root colonization as the mycorrhizal root colonization for AMF and combined inoculation treatments were not different. Similarly, AMF did not influence the percentage of *Trichoderma* root colonization as *T. harzianum*-treated plants; either alone or in combination with AMF, maintained higher root colonization than the control but were not different. The findings indicated that *T. harzianum* and AMF had no suppressive effect on the development of external mycelial growth of each other.

Dry shoot weight was improved by inoculation with AMF and *T. harzianum*, either alone or in combination. *Trichoderma harzianum* and AMF have been found to promote growth and plant development of numerous crops (Altomare et al., 1999; Gravel et al., 2007; Kleifeld &

Chet, 1992; Liu et al., 2008; Samuels, 2006). Chandanie et al. (2009) noted that dual inoculation with *T. harzianum* and AMF synergistically increased the plant dry biomass of cucumber when compared with inoculation of *T. harzianum* alone. Results in this and other studies (Whipps, 1997; Gravel et al., 2007) suggested that *T. harzianum* and AMF improve plant growth development of tomato, probably due to the production of stimulatory compounds and/or the improvement of mineral nutrient availability and uptake.

Results in this study demonstrated that *T. harzianum* and AMF have the potential to improve vegetative growth, fruit quality and early fruit yield of field-grown tomato. However, further investigation is necessary in order to establish whether the rate of microbial colonization could be translated into increased total yield, as these fungi were able to increase early yield. The study did not detect any antagonistic effect between *T. harzianum* and AMF, suggesting that these fungal inoculants could be used in combination to improve the productivity of tomato crop.

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