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Full Length Research Paper

Tomato (*Solanum lycopersicum* L.) seedling growth and development as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

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Recent trends in soil microbiology suggest that certain soil microbes have a positive effect on seedling growth and development. A study was conducted to investigate the interactive effect of the plant-growth promoting fungi *Trichoderma harzianum* and the arbuscular mycorrhizal fungi (AMF) in growth and development of tomato (*Solanun lycopersicum*) seedlings grown under greenhouse conditions. A 3 x 3 factorial experiment was laid out in a completely randomised design with six replications. At harvest (42 DAP), when compared with the control, *T. harzianum* and/or AMF treated plants improved shoot length, root length, dry shoot mass and dry root mass. Pre-inoculation with AMF increased shoot N, P and S content of tomato seedlings, whereas pre-sowing with *T. harzianum* alone increased the shoot N. Generally, shoot Zn and Mn content were affected by both fungi, with the best result observed when AMF was applied 2 weeks after *T. harzianum.* The percentage of roots colonised by AMF was less than 15% regardless of the time when *T. harzianum* was applied. However, the percentage of roots colonised by *T. harzianum* was greater than 90% at all times. In conclusion, this study suggested that *T. harzianum* and AMF have the potential to improve tomato seedling growth and development.

Key words: Essential mineral nutrients, mycorrhiza, plant-growth promoting fungi, seedling quality, *Solanum lycopersicum*.

INTRODUCTION

The need to produce quality tomato seedlings, capable of withstanding adverse abiotic and biotic stresses after transplanting and improve mineral nutrient uptake, inspired producers to consider a combined pre-sowing inoculation of seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF). Nursery inoculation of tomato with AMF resulted in stronger and superior quality seedlings (Giannuzzi et al., 2001), higher crop uniformity (Waterer and Coltman, 1988), better mineral nutrient uptake (Bethlenfalvay et al., 1988; Chandanie et al., 2009; Marschner and Dell, 1994), improved tolerance to soil borne diseases (Pozo and Azcón-Aguilar, 2007), and both reduced stress and increased yields (Chandanie et al., 2009; Lovato et al., 1996). Similarly, *T. harzianum* enhanced plant growth and development (Harman and Taylor, 1990; Liu et al., 2008; Samuels, 2006), and provided protection against soil-borne pathogens that cause damping-off in tomato seedlings (Harman and Taylor, 1990).

The symbiosis between *T. harzianum* and AMF is widely reported in literature (Meyer and Roberts, 2002; Raupach and Kloepper, 1998). *Trichoderma* species have both antagonistic (Camporota, 1985; McAllister et al., 1994;

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Wyss et al., 1992) and stimulating effects on AMF (Calvet et al., 1992; McAllister et al., 1994) and *vice versa*. Antagonistic modes of action of Trichoderma to AMF include competition, myco-parasitism and production of antifungal metabolites (Lorito et al*.,* 1993; Stefanova et al*.,* 1999). Also, the species have a high reproductive capacity estimated at 12 h for spore germination (Liu et al., 2008; Woo et al., 2005). In spite of the increasing interest in the interaction between *T. harzianum* and AMF, information about these interactions in tomato seedling production is scanty (Fracchia et al., 1998; McAllister et al., 1994). The objective of this study was to investigate the interactive effects of nursery inoculation of *T. harzianum* and AMF on their impact on growth and development of tomato seedlings when applied at different times.

MATERIALS AND METHODS

Location

The experiment was conducted under greenhouse conditions at the Hatfield Experimental Farm, University of Pretoria, South Africa, during the 2008 growing season and repeated in 2009. The site is located at 23° 45' S latitude, 28° 16' E longitude, at 1372 m above sea level.

Microbial inoculants

Commercial mycorrhizal inoculum Biocult© containing spores of *Glomus mossae*, was obtained from Biocult Ltd. (Sommerset West, South Africa). Commercial Trichoderma inoculum T-GRO containing spores of *T. harzianum* isolate DB 103 (1×10^9 colony forming units g^{-1}, as a wettable powder) was obtained from Dagutat Biolab (Johannesburg, South Africa). The microbial inoculants were thoroughly mixed with peat moss and vermiculite before applying them into the pasteurised sand: coir (seedling trays) or peat moss (PVC pipe) mixtures used for seedling production. The microbial inoculants were introduced either before sowing the seed or before transplanting the seedlings (two weeks later).

Experimental design and treatments

The nine treatment combinations, namely T_0M_0 (untreated/control), T_0M_1 (treated with AMF only, before sowing), T_0M_2 (treated with AMF only, 2 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, 2 weeks after sowing), T_2M_1 (treated with *T. harzianum* at 2 weeks after sowing and AMF before sowing) and T_2M_2 (treated with both fungi 2 weeks after sowing), were arranged in a completely randomised design with six replications.

Seeds of tomato cv. 'Nemo-Netta' were sown into cell plug trays filled with a pasteurised sand and coir mixture at ratio 50:50 (v/v). Trays were transferred to the germination room for 3 days and then moved to the greenhouse. Two weeks after sowing, seedlings were transplanted into a 30 cm long PVC pipe (diameter: 3.5 cm) filled with peat moss and supported by a cylinder base. Plants were fertilised with half strength modified Hoagland's solution (Spomer et al., 1997) and watered daily.

Data collection

At harvest, 42 days after initiating the treatment, plant height, root length, stem diameter and leaf area were recorded. Roots were separated from shoots and sampled for defeminisation of colonisation with the two fungal species. Roots of randomly selected tomato seedlings were washed free of medium, stained with trypan blue in lactophenol (Phillips and Hayman, 1970) and quantified for percentage of AMF colonisation using the line-intersect method (Brundrett et al., 1996). Root colonisation by *T. harzianum* was also determined (Datnoff et al., 1995).

Shoots and the remaining roots were oven-dried at 50°C for 70 h to determine dry shoot and dry root mass. Dried shoots and roots were each ground in a Wiley mill to pass through 1 mm sieve. 1 g sample was digested in sulphuric acid at 410°C and N determined by an auto analyser. Other essential nutrient elements were digested with a 2:1 nitric/perchloric acid mixture at 230°C and nutrient elements determined by the inductive coupled plasma (ICP).

Data analysis

Data were subjected to analysis of variance using SAS (SAS Institute Inc., Cary, NC, USA. (2002 to 2003). The degrees of freedom and their associated sum of squares were partitioned to provide the total treatment variation for different sources of variation (Little, 1981). Mean separation was achieved using Fisher's least significant difference test. Unless stated otherwise, treatments discussed were different at 5% level of probability.

RESULTS

Root colonisation by fungi

The *T. harzianum* × AMF on root colonisation was not significantly different during both growing seasons (Table 1). Roots of treated *T. harzianum* seedlings had more than 90% root colonisation; AMF-treated seedlings had less than 15% colonisation, whereas untreated roots had no colonisation. Using the partitioning of the degrees of freedom and their associated sum of squares *T. harzianum* contributed 99% to total treatment variation (TTV) in percentage Trichoderma colonisation, whereas AMF accounted for over 96% of the TTV in colonisation.

Growth parameters

This analysis revealed a significant interactive effect of *T. harzianum* and AMF for plant height and root length, which only explained half of the total variability (Table 1). *T. harzianum* contributed ca. 40% of the TTV in the mean plant height. The treatment also explained 21 and 29% of

Table 1. Partitioning of the treatment sum of squares derived from the analysis of variance for the plant growth variables and root colonisation of 6-weeks old tomato seedlings as influenced by *Trichoderma harzianum* and AMF inoculation.

Source	Df	Mycorrhiza		Trichoderma		Plant height		Root length		Dry shoot mass		Dry root mass	
		SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
2008 growing season													
T. harzianum (T)	2	19.7	2.1ns	107215	99.7*	455.39	41.2***	185.27	20.5***	92.01	45.0***	6.95	56.6***
AMF (M)	2	902.48	96.1*	15	0.0ns	87.66	7.9***	260.34	28.8***	32.63	15.9*	0.87	7.1ns
T×M	4	16.74	1.8ns	296	0.3ns	561	50.8***	459.21	50.8***	79.99	39.1**	4.45	36.3*
Total	53	938.92		107526		1104.05		904.82		204.62		12.27	
2009 growing season													
T. harzianum (T)	2	4.59	0.2ns	98415	99.8*	145.67	40.1**	135.38	29.3ns	37.39	81.1***	1.14	78.7*
AMF (M)	2	2013.37	99.3*	104	0.1ns	50.65	13.9ns	70.27	15.2ns	2.11	4.6ns	0.04	2.6ns
T×M	4	10.52	0.5ns	74	0.1ns	167.34	46.0**	256.99	55.5*	6.59	14.3ns	0.27	18.8ns
Total	53	2028.48		98593		363.65		462.64		46.09		1.44	

ns, *,**,*** are levels of significance at P $\geq$ 0.10, P 0.05, P 0.01, P 0.00, respectively.

the TTV in mean root length in 2008 and 2009 growing seasons, respectively. In 2008, AMF contributed 29% of the TTV in mean root length but only 15% during the second growing season. During the first season, inoculating both fungi at sowing (T_1M_1) increased plant height and root length by 40 and 30%, respectively, as compared to the control plants (Table 2). The highest plant height was obtained with late *T. harzianum* inoculation (T_2M_0). In 2009, the highest plant height and root length were recorded with T_1M_1 and T_2M_0, respectively, whereas the lowest counts were obtained in the untreated plants (T_0M_0). In both seasons, all the microbial inoculated seedlings, except for late microbial inoculations (T_2M_2), when compared with the control increased plant height and root length.

Biomass production

There was a significant *T. harzianum* × AMF effect on dry shoot and root mass during the first growing season, which accounted for ca. 40% of the TTV of dry shoot mass. The major source of variability was due to *T. harzianum*, which contributed nearly 50% of the TTV of dry shoot mass. Interestingly, in 2009, *T. harzianum* accounted for ca. 80% of the TTV with small contributions from AMF and *T. harzianum* × AMF interactions. During the first season, compared to the control plants, the combined inoculation of *T. harzianum* and AMF before sowing resulted in 35% higher dry shoot mass, whereas inoculating both fungi simultaneously 2 weeks after sowing, resulted only in 13% increase. The highest increase (52%) in dry shoot mass was obtained with T_1M_0. All microbial inoculants increased dry shoot mass (Table 2). Dry root mass was increased (up to 37%) when *T. harzianum* was inoculated before planting and AMF 2 weeks later (T_1M_2).

However, a negative interaction between *T. harzianum* and AMF was observed when both fungi were applied 2 weeks after sowing (T_2M_2), resulting in the lowest dry root mass. During the second season, irrespective of the AMF treatment, inoculating *T. harzianum* before sowing increased the dry mass of the shoot and root by 19 and 11%, respectively, whereas dry shoot and root mass in plants inoculated with *T. harzianum* 2 weeks later, did not differ from those of the control.

Table 2. Plant growth variables of 6-week old tomato seedlings as influenced by *Trichoderma harzianum* and AMF inoculation.

Treatment	Plant height (cm)			Root length (cm)			Dry shoot mass (g plant^{-1})			Dry root mass (g plant^{-1})		
	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2
2008 growing season												
T_0	16.73f	25.12c	21.40e	22.38e	33.74a	29.28bc	6.00d	8.80bc	6.93cd	1.89b	2.46ab	1.94b
T_1	27.34b	28.11ab	28.56a	26.63d	34.23a	32.66a	12.50a	9.17bc	9.36bc	2.91a	2.83a	2.98a
T_2	29.16a	23.08d	17.15f	29.86b	26.92cd	23.21e	10.31ab	6.91cd	6.89cd	2.89a	1.91ab	1.84b
2009 growing season												
T_0	20.25d	26.52ab	25.15bc	21.82c	28.88ab	26.33bc	8.24d	9.33bcd	8.71cd	2.47c	2.67abc	2.50bc
T_1	27.07ab	29.30a	27.31ab	27.80ab	29.68ab	30.10ab	10.58ab	10.70ab	10.80a	2.79abc	2.90ab	2.92a
T_2	27.47ab	25.30bc	22.66cd	31.75a	30.00ab	24.82bc	9.75abc	9.46abcd	8.54cd	2.69abc	2.54abc	2.51bc

T_1, T_2 and T_0: *T. harzianum* inoculated before sowing, 2 weeks after sowing or uninoculated. M_1, M_2 and M_0: AMF inoculated before sowing, 2 weeks after sowing or uninoculated. Column means followed by the same letter were not significantly different at 5% level according to Fisher's least significant different test.

Shoot chemical analysis

Neither *T. harzianum* nor AMF affected essential nutrient elements such as K, Ca, Mg, Mo and Na. There was a significant *T. harzianum* × AMF interaction term for the shoot Mn and Zn content, whereas P and S were only affected by AMF. Mean shoot N content of seedlings was affected by *T. harzianum* and AMF, but not their interaction (Table 3).

Inoculating *T. harzianum* before sowing (T_1) increased the N shoot content by 6%, whereas later inoculation was similar to the uninoculated plants (T_0). On the other hand, when compared with the control (M_0), inoculating AMF before (M_1) or 2 weeks after sowing (M_2) increased the shoot N content by 9 and 10%, respectively (Table 4).

Inoculating AMF before (M_1) or after sowing (M_2) increased the shoot P content of tomato seedlings by ca. 18 and 16%, respectively. Shoot S increased by 15% when AMF was inoculated before sowing (M_1), whereas later inoculation (M_2) had no effect on this nutrient element (Table 4). Inoculating *T. harzianum* and AMF before (T_1M_1) or after (T_2M_2) sowing increased Mn content by 18 and 9%, respectively. However, the highest Mn shoot content increase (33%) was obtained with a combination of early *T. harzianum* and late AMF application (T_1M_2) (Table 5). Similarly, for Zn shoot content, the highest increase (34%) was recorded with T_1M_2, while T_1M_1 and T_2M_2 yielded about 13 and 10% Zn increase, respectively.

DISCUSSION

Nursery inoculation of tomato with *T. harzianum* and AMF improved most of the growth variables of tomato seedlings, increased nutrient element uptake and permit microbial root colonisation. Uninoculated plants showed no Trichoderma or AMF or colonisation, indicating that these fungi were not indigenous to the specific growth media. The low mycorrhizal colonisation (< 15%) observed was in agreement with Chandanie et al. (2009), who argued that the 13% level of colonisation with AMF observed before transplanting should be considered adequate for successful establishment of mycorrhizal seedlings. According to Bierman and Linderman (1983), less than 13% root colonisation should not be a concern, as these fungi would spread rapidly to new roots after transplanting. On the other hand, the higher Trichoderma root colonisation was due to its high reproductive capacity (Woo et al., 2005).

Observations in this study suggested that, low mycorrhizal and high Trichoderma root colonisations were due to their inherent individual abilities to colonise tomato roots rather than their

Table 3. Results of ANOVA (P values) executed for the shoot mineral nutrient content for the 2008 growing season on tomato seedlings at 42 days after planting.

Response variables	N	P	K	Ca	Mg	S	Mn	Zn	Cu	Mo	Na
T (df = 2)	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M (df = 2)	**	*	ns	ns	ns	*	*	*	ns	ns	ns
T×M (df =4)	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns

ns, *,**,*** are levels of significance at P $\underline{>}$ 0.10, *P* 0.05, *P* 0.01, *P* 0.00, respectively. T = *T. harzianum*; M= AMF.

Table 4. Macronutrients shoot content of 6- week old tomato seedlings as influenced by *T. harzianum* and AMF applied before sowing and at two weeks after sowing.

Response variable	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
T (*T. harzianum*)						
T_0	4.42b	0.62	2.97	4.19	1.06	1.63
T_1	4.72a	0.63	2.75	4.17	1.03	1.56
T_2	4.45b	0.60	2.72	4.48	1.13	1.77
M (AMF)						
M_0	4.23b	0.54b	2.80	4.00	1.05	1.57b
M_1	4.65a	0.66a	2.86	4.47	1.13	1.83a
M_2	4.71a	0.64a	2.77	4.37	1.05	1.56b

T_1, T_2 and T_0: *T. harzianum* inoculated before sowing, 2 weeks after sowing or uninoculated. M_1, M_2 and M_0: AMF inoculated before sowing, 2 weeks after sowing or uninoculated. Column means followed by the same letter were not significantly different at 5% level according to Fisher's least significant different test.

Table 5. Micronutrient shoot contents of 6-week old tomato seedlings as influenced by AMF pre-inoculation.

T_1, T_2 and T_0: *T. harzianum* inoculated before sowing, 2 weeks after sowing or uninoculated. M_1, M_2 and M_0: AMF inoculated before sowing, 2 weeks after sowing or uninoculated. Column means followed by the same letter were not significantly different at 5% level according to Fisher's least significant different test.

competitive interactions. However, the observation was not in agreement with McGovern et al. (1992) who reported antagonistic effect of *Trichoderma* on AMF in tomato. Chandanie et al. (2009) observed a decreased *T. harzianum* growth due to AMF inoculation in cucumber (*Cucumis sativus*).

However, Green et al. (1999) observed a mutually inhibitory interaction between *T. harzianum* and the external mycelia of an AMF *Glomus intraradices*. Apparently, the interaction between Trichoderma and AMF is species and host-plant specific (Fracchia et al., 1998; Green et al., 1999; Rousseau et al., 1996).

Trichoderma harzianum and AMF, either inoculated alone or in combination, increased the root length and plant height of tomato. Generally, improved plant growth had been observed under *Trichoderma* (Duffy et al., 1997; Ozbay and Newman, 2004) and AMF inoculations (Tahat et al., 2008). Improved plant growth observed in these experiments might be due to increased solubility of insoluble plant nutrients by *Trichoderma* species (Kaya et al., 2009) or enhanced immobile nutrient elements uptake by AMF (Bethlenfalvay et al., 1988; Chandanie et al., 2009; Marschner and Dell, 1994) . Observations in this study suggested that, there were the desired beneficial effect of nursery inoculation with *T. harzianum* and/or AMF on dry matter production of tomato seedlings, which

was in agreement with Ozbay and Newman (2004), who observed an increased dry shoot mass due to Trichoderma inoculation and Tahat et al. (2008) who observed similar trends with AMF. Chandanie et al. (2009) demonstrated that, the combined inoculation of AMF with Trichoderma synergistically increased dry shoot mass when compared with inoculation of Trichoderma and AMF alone. McAllister et al. (1994) reported a decrease in dry shoot mass when Trichoderma was inoculated alone before sowing or at the same time with AMF. In this study, both fungi either applied alone or in combination, improved plant growth, except when simultaneously applied 2 weeks after sowing. The negative interaction when combined inoculation is applied at 2 weeks could be due to competition for nutrients or space.

Nursery microbial inoculation had no effect on K, Ca and Mg shoot content, which was in agreement with Karagiannidis et al. (2002), who did not find any positive effect of mycorrhiza on shoot K and Ca content. Increased K and Mg content have been reported in wheat inoculated with AMF (Tarafdar and Marschner, 1995), whereas *Trichoderma* species did not increase the shoot Ca, K and Mg content in tomato seedlings grown in hydroponics (Yedidia et al., 2000). Nevertheless, there were beneficial effects of AMF inoculation on shoot N, P and S in tomato seedlings. Increased N uptake due to AMF inoculation had been reported previously (Karagiannidis et al., 2002; Thomson et al., 1996). Similarly, increased shoot P contents following AMF inoculation were in agreement with other observations (Al-Karaki, 2006; Nurlaeny et al., 1996; Yedidia et al., 2000), whereas others did not observe any positive effect (Inbar et al., 1994). Late inoculation had no effect on shoot S, suggesting that early application was advisable for increased S uptake. Increased S content of plants with mycorrhiza had been reported previously (Rhodes and Gerdemann, 1978).

Shoot Zn and Mn increased in nursery inoculation, probably due to an increased absorptive capability for these nutrient elements when tomato roots are colonised by *Trichoderma* and AMF, as suggested for pepper plants (Kaya et al., 2009). However, this is in disagreement with a reduced concentration of Mn and Zn on leaves of AMF infected plants (Weissenhorn et al., 1995). Other micronutrients such as Cu, Mo and Na were unaffected by the nursery microbial inoculation, possibly due to their low concentration in the growing medium.

Conclusion

Nursery inoculation of tomato with *T. harzianum* and/or AMF improved growth and development of tomato seedlings. *T. harzianum* and AMF synergistically improved most of the growth variables in tomato seedlings. A negative *T. harzianum* × AMF interaction was only observed 2 weeks after sowing, probably due to competition for nutrient elements and/or infection sites. In contrast to *T. harzianum*, which had little effect on essential nutrient elements, AMF inoculation affected the nutrient uptake of key elements such as N, P, S, Zn and Mn. Although, the myco-parasistic effect of *Trichoderma* species is well known, results of this study demonstrated that, this plant-growth promoting fungi can successfully be inoculated with AMF for improved seedling health and development in tomato production.

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