INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–119/DAK/2010/12–5–789–792
http://www.fspublishers.org

Full Length Article

Effect of Arbuscular Mycorrhiza (AM) Inoculation on the Performance of Tomato Nursery Seedlings in Vermiculite

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ABSTRACT

A greenhouse experiment was conducted to evaluate the responsiveness of tomato (*Lycopersicon esculentum* L.) seedlings to arbuscular mycorrhizal inoculation in a soilless medium on the transplant production performance. The tomato variety RODADE was inoculated with BIOCULT mycorrhiza granules (consisting of both *Glomus etunicatum* & *G. intraradices*) and hydroponically grown on vermiculite for six weeks. Measurements on the tomato seedlings taken at 14, 28 and 42 days included shoot and root fresh weight and dry weight, leaf area, stem length, leaf area ratio (LAR), relative growth rate (RGR), unit leaf rate (ULR), mycorrhiza dependency (MD) and growth response to arbuscular mycorrhiza (AM) fungi root inoculation. AM fungi inoculated seedlings exhibited better transplant performance due to its higher shoot fresh weight (avg. 11.28 g plant^{-1}), high shoot/root ratio (avg. 0.236), higher root biomass (avg. 2.17 g plant^{-1}) higher RGR (7.34 mg g^{-1} day^{-1}) and ULR 1.28 mg cm^{-1}day^{-1}). AM inoculation resulted in 23.3% root colonization by the fungi in the vermiculite medium. The use of AM fungi appeared to provide benefits to the development of tomato seedling transplants in a soilless nursery condition and might be of particular interest under organic farming conditions. © 2010 Friends Science Publishers

Key Words: Arbuscular mycorrhiza; Biocult; *Lycopersicon esculentum*; Nursery; Swaziland; Vermiculite

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligatory biotrophic symbionts occurring in nearly all natural and agricultural soils and commonly colonize roots of many plant species (Smith & Read, 1997). The primary effect of AMF on their host plant is an increase in plant growth and nutrient uptake (Ortas *et al*., 2001). Plants with mycorrhiza are potentially more effective at nutrient and water acquisition (Koide, 1991) and less susceptible to disease (Pfleger & Linderman, 1994). They can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan & Angus, 2003) and consequently, increase root and shoot biomass and improve plant growth. Vegetable crops that require a nursery stage can benefit from AMF inoculation, thus its use has been incorporated into horticultural practices (Evans, 1997). Most horticultural nurseries now prefer the soilless production of crop seedlings for the purpose of high yield and good quality crops compared to traditional greenhouse production in soil. In contrast to native ecosystems, where mycorrhizas are so common, soilless mixes used in nurseries for plant propagation do not contain propagules of mycorrhizal fungi (Azcón-Aguilar & Barea, 1996) and mycorrhizal inoculation in soilless media has been found to increase crop uniformity, reduce transplant mortality and increase productivity of different horticultural crops (Vosatka *et al*., 1999; Ikiz *et al*., 2009). Presently, the use of AMF application as a biofertilizer has been recommended with the aim of increasing productivity and reducing fertilizer use.

Tomato is recognized as a mycotrophic plant (Kubota *et al*., 2005) and the usefulness of AMF inoculation in improving the fitness and vitality of tomato host has been described under stress conditions (Karagiannidis *et al*., 2002). Muchovej (2004) reported that application of AVM reduce the susceptibility of tomato roots to soil borne pathogens such as *Fusarium* spp, *Rhizoctonia solani*, which cause seedling damping-off in the nursery. Literature on the potential effects of AMF on the growth of nursery seedlings and yield of vegetable crops in soilless medium are very scanty, nonetheless recent studies have shown that this might be of a great importance for the advancement of vegetable production, particularly organic-biological production. To incorporate mycorrhizal technology in nursery conditions, the mycorrhizal associations are three way interactions between plants, fungi and growing media (Brundrett *et al*., 1996), since the effectiveness of a particular mycorrhizal fungus varies with the plant and the growing conditions (Corkidi *et al*., 2004). Thus, the successful application of mycorrhizal inoculums is related to the choice of potting mixes.

The present investigation was undertaken to determine the effect of mycorrhizal inoculation on the performance of cultivated tomato seedlings in a soilless nursery medium

To cite this paper: Oseni, T.O., N.S. Shongwe and M.T. Masarirambi, 2010. Effect of arbuscular mycorrhiza (AM) inoculation on the performance of tomato nursery seedlings in vermiculite. *Int. J. Agric. Biol.*, 12: 789–792

with a view to exploit it in the production of quality seedlings for sustainable organic vegetable production in Swaziland.

MATERIALS AND METHODS

This experiment was carried out in the year 2009 (from mid-January to mid-April) in a greenhouse of the Horticulture Department, Faculty of Agriculture, Luyengo Campus of the University of Swaziland. The site is located at Luyengo, Manzini Region in the Middleveld agro-ecological zone of Swaziland. Luyengo is 26^o 34’ S and 31^o 12’ E. The average altitude of this area is 750 m above sea level. The mean annual precipitation is 980 mm with most of the rain falling between October and March (Anonymous, 2006). Tomato seeds (*Lycopersicon esculentum* L. Var. RODADE from Starke Ayres seeds Company, South Africa) were washed under running tap and then submerged in 70% alcohol for 30 s.

The seeds were then transferred into a beaker containing 40% sodium chloride and 3 drops of Tween 20 and stirred for 10 min. The sterilant was decanted and seeds rinsed several times with distilled water and then germinated in a large tray containing moist sterilised vermiculite for 4 weeks. To produce mycorrhizal plants, uniform seedlings were selected and each transplanted into plastic pots (15.0±2 cm diameter) filled with 2.0±0 kg (air-dry) vermiculite and approximately 0.50 mg of Biocult granules were placed in the planting holes around the roots at the time of transplanting and a total of 30 pots were inoculated with the Arbuscular mycorrhiza species (BIOCULT *granules*) consisting of both *Glomus etunicatum and G. intraradices* [BIOCULT (Pty.) Ltd, South Africa]. Similarly, another 30 pots of non inoculated plants (control) were prepared and all treatments were arranged in a randomized complete block design on the greenhouse benches and replicated three times.

The plants were grown in the greenhouse under natural photoperiods (23.5˚/18˚C) day/night, 4000-6000 lux light intensity) for 6 weeks during, which only distilled water was applied. Three plants from each treatment were randomly selected and harvested at three different periods (14, 28 & 42 days) after AMF inoculation. During each harvests, plants were separated into leaves, shoots and roots, weighed to determine the fresh weight and then dried at 70^{o}C for 48 h for the dry weight determination. The leaf area, plant dry and fresh weights were used as a measure of growth by calculating the leaf area ratio (LAR), relative growth rate (RGR) and unit leaf rate (ULR) using the classical growth analysis methods (Hunt, 1982). Relative mycorrhizal dependency (RMD) of tomato seedlings was calculated by expressing the difference between shoot dry weight of the mycorrhizal plant and the shoot dry weight of the non mycorrhizal plant as a percentage of the shoot dry weight of the mycorrhizal plant (Plenchette *et al*., 1983). The roots were cleared and stained by using the methods by Phillips and Hayman (1970) and the percentage of mycorrhizal colonization was estimated by the methods of Brundrett *et al*. (1984).

Statistical analysis of the data were done using analysis of variance (ANOVA) and F-test was used to evaluate treatment significance at $P<0.05$ according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

In general, AMF inoculation caused measurable changes in growth parameters recorded on tomato nursery seedlings at 14, 28 and 42 days, though some were not significantly different from non-inoculated control treatments (Table I). These findings agreed with literature reports of previous studies in soil and soilless systems with different plant species such as in *Eucalyptus camaldulensis* (Misbahuzzaman & Ingleby, 2005), gladiolus (Javaid & Riaz, 2008), pepper (Ikiz *et al*., 2009), onions (Goussous & Mohammad, 2009) and vegetable crops (Matsubara *et al*., 1994), where AMF inoculation produced significantly higher plant growth compared to the untreated control. Bio-augmentation with native Arbuscular mycorrhiza fungi was also reported to improve the qualities of seedlings in nurseries (Bagyaraj *et al*., 1989). Leaf number and leaf size, which plays an important role in seedling growth and development were not significantly affected in both treatments, although inoculated tomato seedlings exhibited higher values of leaf number and leaf area compared to control (Table I).

The fresh root weight was significantly higher in inoculated seedlings compared to non-inoculated control at 14, 28 and 42 days after AMF inoculation, which might probably enhance uptake of nutrients and better growth in the field (Table I). Wang *et al*. (2008) reported that inoculation at initial stage of plant development can promote AM symbiosis, which leads to increase in plant growth in the nursery and improving performance after planting in the field. The mean fresh biomass weight of inoculated tomato (11.28 g plant^{-1}) was higher but not significantly different from the control (9.58 g plant^{-1}). Similar trend was observed in stem length. However, both stem length and total fresh biomass were significantly higher in the inoculated seedlings at 28 days compared to the control, but not at 14 or 42 days after inoculation (Table I). This probably indicated that AMF inoculation on tomato seedlings was optimally effective at this stage in promoting higher vegetative growth of the seedlings. Bryla and Koide (1998) reported higher values for growth parameters in AMF inoculated tomato in comparison to other treatments at 31days after transplanting and opined that changes in responsiveness to mycorrhizal colonization occurred with plant developmental stages in tomato. Similarly, Javaid and Riaz (2008) also reported enhanced vegetative growth in Gladiolus varieties inoculated with Arbuscular mycorrhiza fungi at early vegetative stage of growth.

Table I: Influence of AMF inoculation on the growth parameters of tomato seedlings

Treatment	Stem length (cm)	Leaf area (cm^2)	Leaf number	Fresh shoot weight (g)	Fresh root weight (g)	Total fresh biomass (g)
			14 Days			
No AMF	9.43^{a}*	6.31^{a}	4.41^{a}	6.78b	1.07^{b}	7.85^{a}
+ AMF	9.99^{a}	9.11^{a}	6.13^{a}	8.29a	1.58^{a}	9.87^{a}
			28 Days			
No AMF	11.67^{b}	15.88^{a}	6.30^{a}	8.08a	1.65^{b}	9.73^{b}
+ AMF	13.98^{a}	20.61^{a}	8.92^{a}	9.11a	2.27^{a}	11.38^{a}
			42 Days			
No AMF	13.33^{a}	19.43^{a}	7.11^{a}	9.03a	1.92^{b}	10.95^{a}
+ AMF	14.51^{a}	25.07^{a}	9.69^{a}	9.94a	2.65^{a}	12.59^{a}
			Overall Means			
No AMF	11.48^{a}	13.87^{a}	5.94^{a}	8.03^{a}	1.55^{b}	9.58^{a}
+ AMF	12.49^{a}	18.26^{a}	8.25^{a}	9.11^{a}	2.17^{a}	11.28^{a}

Table II: Influence of AMF inoculation on the dry biomass parameters and root/shoot ratio of tomato seedlings

Treatment	Shoot dry weight (g)	Root dry weight (g)	Total dry biomass (g)	Fresh root/shoot ratio
		14 Days		
No AMF	1.98^{a}	0.64^{b}	2.62^{b}	0.158^{b}
+ AMF	2.31^{a}	0.98^{a}	3.29^{a}	0.191^{a}
		28 Days		
No AMF	2.32^{b}	0.99^{b}	3.31^{b}	0.199^{b}
+ AMF	2.88^{a}	1.46^{a}	4.34^{a}	0.249^{a}
		42 Days		
No AMF	2.37^{b}	1.08^{b}	3.45^{b}	0.236^{b}
+ AMF	3.17^{a}	1.54^{a}	4.71^{a}	0.267a
		Overall Means		
No AMF	2.22^{b}	0.90^{b}	3.13^{b}	0.198^{b}
+ AMF	2.79^{a}	1.33^{a}	4.11^{a}	0.236^{a}

The shoot, root and total dry biomass of tomato seedlings increased significantly with AMF inoculation as compared to control at 14, 28 and 42 days after inoculation (Table II). The total dry biomass of AMF inoculated seedlings increased by 31.31% in comparison to the control, which is indicative of potential better performance in the field. The trends of both fresh and dry root biomass were similar, being significantly higher in inoculated compared to non-inoculated seedlings. Thus, the capacity of the seedlings to overcome transplanting shock will depend on the capacity of the roots to support structural and functional changes, absorption of water and nutrients as well as the capacity of regenerating new roots (Scagel, 2004). The root/shoot ratio varied significantly amongst the different treatments, but inoculated tomato seedlings had significantly higher root/shoot ratios than the non-inoculated seedlings at 14, 28 and 42 days after AMF inoculation (Table II). This indicated that AMF inoculated seedlings partitioned more biomass to the roots than shoots resulting in higher root/shoot ratio compared to the non-inoculated seedlings. Tobar *et al*. (1999) reported that higher root/shoot ratio reflected a high degree of mycorrhiza effectiveness. An improvement in the quality of seedlings observed in terms of growth parameters as compared to control further strengthened that the inoculated seedlings improved their vigour, robustness and development and consequently transplanting performance. This beneficial effect of AMF on horticultural crops was reported in pepper inoculated with G. *intraradices* (Aguilera-Gomez *et al*., 1999). In soilless substrates lacking mycorrhizal fungi, mycorrhizal inoculation has been found to increase crop uniformity, reduce transplant mortality and increase productivity of different horticultural crops (Vosatka *et al*., 1999).

The physiological responses of AMF inoculated tomato seedlings measured in terms of RGR and ULR averaged over the harvesting periods were significantly higher than the non-inoculated seedlings (Table III). Similarly, the LAR of inoculated seedlings was higher but not significantly different from the non-inoculated seedlings. The photosynthesis activity measured in terms of unit leaf rate, which increased as a result of AMF inoculation, had a close relationship with relative growth rate thereby producing significantly bigger seedlings than the non-inoculated control. Wu and Xia (2006) reported increased rate of photosynthesis with mycorrhiza association in citrus seedlings. Therefore, AM fungi may function as a metabolic sink causing basipetal mobilization of photosynthates to roots thus, providing a stimulus for greater photosynthetic activity (Wu & Xia, 2006). The mean value of mycorrhiza dependency (MD) calculated from shoot dry matter weight was 17.76% in the AMF inoculated seedlings and this measures the seedling growth responses to AMF colonization. This result confirms the high dependency of tomato on mycorrhiza. In his review, the mycorrhiza dependency of tomato was found to be 59% (Tawaraya, 2003). At 42 days after inoculation, no AMF colonization was found on the roots of control seedlings, while] in the inoculated, the percentage root colonization was 23.30% (Table III). Akond *et al*. (2008) reported an average of 35.99% mycorrhiza colonization of tomato roots in soils. However, high AMF infectiveness does not always guarantee an improvement in plant growth (Carpio *et al*., 2005). It therefore, appears that the main effects of mycorrhiza root colonization on tomato seedlings growth could be attributed to mycorrhiza mediated increases in ULR and RGR resulting in high fresh and dry biomass production. The overall growth response obtained in this study was 25.63% in AMF inoculated seedlings compared to non-inoculated seedlings in the soilless medium.

Table III: Effect of AMF inoculation on LAR, RGR, ULR, MD and % colonization and growth response of tomato seedlings

Treatment	LAR (cm g^{-1})	RGR (mg g^{-1} day^{-1})	ULR (mg cm^{-2} day^{-1})	Mycorrhizal Dependency	%AMF Colonisation	% Growth response
No AMF	5.299[a]*	0.858[b]	0.162[b]	0. 00[b]	0.00[b]	0.00[b]
+ AMF	5.702[a]	7.341[a]	1.282[a]	17.76[a]	23.30[a]	25.63[a]

*Data in the same column followed by the same letter are not significantly different (P <0.05)

CONCLUSION

AMF inoculating tomato seedlings did not promote significant seedling growth but positively affected growth and development resulting in higher biomass dry weight and root/shoot ratio with beneficial implications for tomato growth and yield. It is important to determine the AMF strain and the ecotype of the crop when carrying out such studies. AMF strain (Biocult) enhanced tomato seedling growth in the vermiculite in the absence of nutrient application. Therefore, application of AMF in horticulture is an effective biotechnology for improving growth and thus expected to be of greater benefits in organic vegetable production.

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(Received 20 March 2010; Accepted 11 May 2010)