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Selection of Arbuscular Mycorrhizal Fungi Species for Tomato Seedling Growth, Mycorrhizal Dependency and Nutrient Uptake

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Summary

Arbuscular mycorrhizae (AM) associations are of great importance in horticultural seedling quality in the Mediterranean region, but information on host dependence and host responsiveness to the fungi species is scarce. Thus, this study was designed to assess the effects of several mycorrhizal fungi species and their cocktail on tomato (*Solanum lycopersicon* L.) seedling growth, quality and nutrient uptake.

Tomatoes were grown in growth medium and inoculated with *Glomus (G.) mosseae*, *G. clarum*, *G. etunicatum*, *G. intraradices*, *G. caledonium* and cocktail of them. The experiments were carried out three successive years from 1999 to 2001. Plants were harvested once in the first year experiment, but in the second and third year experiments plants were harvested twice at different developmental stage. In experiments, non-inoculated, seed inoculation and seedling inoculation were compared.

Pre-inoculation and re-inoculation increased growth parameters of tomato seedlings in three years. The inoculated plants produced significantly higher biomass than those of non-inoculated plants. In general, plants re-inoculated at seedling stage had higher growth performance than seed inoculated plants. However pre-inoculation (seed stage inoculation) have higher mycorrhizal dependency than re-inoculation (seedling stage). All the inoculated plants were extensively colonized by all of the AM fungi species. Inoculated plants flowered earlier than non-inoculated plants. The AM plants generally enhanced content of phosphorus (P) and zinc (Zn) and the *G. etunicatum* inoculated plants particularly had higher nutrient uptake compared to the other AM plants. Plant response to the mycorrhizal inoculation was different for both inoculation times. There were no specific species showed superiority to others for both inoculation times and three years repetition, however in generally, *G. clarum* mycorrhiza species gave the highest responses as regarded to measured parameters.

Key words. AMF species – flowering – mycorrhiza – P and Zn nutrient content – seedling development

Introduction

Arbuscular-mycorrhizal fungi (AMF) have been shown to improve crop productivity of numerous crop plants including horticultural plants in soils with low fertility (Plenchette et al. 1983; Jeffries 1987; Ortas and Varma 2007; Ortas 2010, 2012; Ortas et al. 2011a, b). Colonization of roots by AMF increases the uptake of nutrients and allows plants to thrive in soils with low levels of nutrient availability (Smith and Read 2008). Sharma et al. (2004) reported that colonization of the root system by AMF confers benefits directly to the hosts plant growth and development by increasing nutrient uptake and also improves plant tolerance to stress conditions. This response is usually attributed to enhanced uptake of less mobile nutrients such as P and Zn (Faber et al. 1990; Fageria and Baligar 1997; Clark 2002; Cavagnaro et al. 2006; Cavagnaro 2008; Cimen et al. 2010; Ortas 2010; Latef and He 2011). In soils with adequate levels of these nutrients, mycorrhizal fungi may not contribute significantly to plant growth (Jeffries 1987; Sari et al. 2002). However, accumulating evidence suggests that colonization by AM fungi may also improve the drought resistance of crop plants (Fageria and Baligar 1997; Sylvia et al. 1993). The increase in nutrient uptake and drought resistance may be related to increase in root length, depth and development of external hyphae (Faber et al. 1990). It has been demonstrated that AMF inoculated tomato plants grow better than non-inoculated plants under salt-stress conditions (Ozdemir et al. 2010; Demir et al. 2011). Karagiannidis et al. (2002a)

reported that mycorrhizal inoculation increased plant growth, root infection, N and P uptake, and also improved the fitness and vitality of tomato. 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 (Oseni et al. 2010; Ren et al. 2010; Dennett et al. 2011; Nzanza et al. 2011). Mycorrhizae inoculated tomato plants fruits are uniform than non inoculated plants (Waterer and Coltman 1988; Dasgan et al. 2008). Sylvia and Chellemi (2001) and Dennett et al. (2011) reported that reduced P application may allow tomato plants to take advantage of their inherent responsiveness to mycorrhizae in a low to moderate soil-P environment.

As AMF has potential affection on the growth of nursery seedling and yield of vegetable crops, this may be important for organic vegetable production as well. Since soils in the Mediterranean regions are P and Zn deficient, mycorrhizal inoculation may help plants to grow (Ortas 2012). The aim of this study was to search the possibility of using mycorrhizal species inoculum as organic sources for increasing tomato seedling quality, growth and nutrient uptake under seed and seedling stages of inoculation.

Material and Methods

AMF material

The AMF isolates used in this study were 1) *Glomus etunicatum* (Becker and Gerdemann), 2) *G. clarum* (Nicolson and Schenck) supplied from Nutri-Link Isolate, Nutri-Link Ltd from United Stadet, 3) *G. intraradices* (Schenck and Smith), 4) *G. caledonium* (Nicolson and Gerdemann), and 5) *G. mosseae* (Nicolson and Gerdemann) were supplied from Rothamsted Experimental Station, UK, and 6) a cocktail (a mixture of the five AMF species in equal portions). Inoculum of each AMF isolate was previously multiplied in the greenhouse using sudangrass [*Sorghum bicolor* (L.) Moench] as host plants grown in Andezitik tuff, soil and compost (7:2:1 v/v) mixture.

Experimental design

The experiments were carried out from 1999 to 2001 under greenhouse conditions. The experiment was designed in a randomized complete block design for six mycorrhizae species, two inoculum stages and two harvests with six replications. They were performed in two stages. In the first stage inoculated seedlings were produced. In the second stage half of the seedlings were re-inoculated with the same sources of AMF species. Seed sowing time for 1999, 2000 and 2001 is 01/04/1999, 09/03/2000, and 17/03/2001 respectively. Plant harvest time for 1999 is 10/07/1999, for year 2000 and 1st harvest is 01/07/2000, for 2nd harvest is 15/06/2000. For year 2001 and 1 st harvest is 7/06/2001 and for 2 nd harvest is 21/06/2001.

Five weeks after seed sowing, inoculated and non-inoculated uniform seedlings at two true-leaf stages with the AMF species were transplanted into 2 L plastic pots. One seedling was transplanted to each pot. In order to make clear the inoculum quantity and use of time, half of the seedlings were re-inoculated with the same mycorrhizae species by adding 500 spores from each mycorrhizae species to the growth medium 30 mm below the seedling roots to investigate the effect of mycorrhizal inoculation at seedling stage on plant growth before the seedlings were transplanted. Non-mycorrhizal seedlings were transplanted to equal amounts of mycorrhizae free growth medium. Distilled water was added daily to maintain moisture of the medium near the 75 % of field capacity.

Tomato seedlings

Tomato (*Lycopersicon esculentum* Mill., cv. 'SC2121') seedlings (plantlets) were produced under greenhouse conditions with and without mycorrhizal inoculation for three successive years. The tomato seeds were surface-sterilized before sowing by shaking in a sodium hypochlorite solution (1 % available chlorine) for 10 min, rinsed three times and then soaked in distilled water several times. Mycorrhizae fungi species *Glomus mosseae*, *G. etunicatum*, *G. intraradices*, *G. clarum*, *G. caledonium* and Cocktail were used. Seeds were sown for 5 weeks in trays to produce seedlings. Uniform seedlings were transplanted into 2 L plastic pots at 2 true-leaf stages. Pots were surface-sterilized with ethanol prior to filling, and then filled with an Andezitik tuff, soil and compost mixture (7:2:1 v/v) growth media.

Growth medium

Soil was collected from 0–20 cm depth in Canakcı soil series (Typic Xerofluandnts) in the Çukurova Basin. The soil is clay loam, pH is 7.52 and Olsen extractable P is 3.8 mg kg^{-1} soil. DTPA extractable Zn, Fe, Cu and Mn is 0.42, 3.84, 1.44, and 2.24 respectively. The growth medium was sterilized at 120 °C for 2 h to eliminate any microorganisms that could affect the function of the arbuscular mycorrhizae.

Plant analysis and mycorrhizal colonization

At seed and seedling stages harvest total dry weight of roots and shoots were recorded for each plant. All the shoots were separated from roots 0.5 cm above the soil surface. At harvesting shoots and roots were thoroughly washed with distilled water and dried at 70 °C for 48 h. Then 0.2 g of the ground plant materials were ashed at 550 °C followed by dissolution in 3.3 % HCl. After the digestion of the plant material, the content of P was determined by the (Murphy and Riley 1962) method by using a spectrophotometer. Zn content was determined by an atomic absorption spectrophotometer.

Before drying the roots, small sub samples were taken and preserved in a mixture of ethanol, glacial acetic acid and formalin, for determination of mycorrhizal infection. Small portions of preserved roots (1 g.) were stained by the method of (Koske and Gemma 1989) and root colonization was determined using a gridline-intersect method according to (Giovannetti and Mosse 1980).

Statistical analysis

Data were analyzed using analysis of variance procedures (ANOVA) and differences among treatments and means were compared using Duncan's Multiple Range Test at 5 % significance level by the SAS (SAS 2009).

Results

Experiment I (Year 1999)

In 1999, the effect of different AMF species inoculation increased tomato plant biomass, shoot and root dry weight (Fig. 1). *G. mosseae*, *G. caledonium* and *G. clarum* species were the more efficient species. AMF species significantly increased shoot dry weight (SDW) ($P < 0.05$) but inoculation times statistically did not make any significant contribution (Table 1). In seed stages *G. intraradices* inoculated plants produced 29 g plant^{-1} SDW but non-inoculated control plants produced 20 g plant^{-1}. Cocktail mycorrhiza also produced less SDW. In seedling stages, *G. caledoinum* and Cocktail mycorrhizal inoculation produced high SDW (Fig. 1). Root dry weight (RDW) has similar growth trend.

The effects of mycorrhizal inoculation on flowering times per cluster were investigated and it was found that in seed inoculation *G. mosseae*, *G. intraradices* and *G. caledonium* were the effective species for each inoculation time (Table 2). However in seedling inoculation stages *G. caledonium* was the most effective one.

Mycorrhizal tomato plants flowered 8 days earlier than non-mycorrhizal plants. Although there was a small difference between seed and seedling inoculation, seedling inoculated plants seems to grow better than non-inoculated plants.

Plant P content was analyzed and in both seed and seedling inoculated plants P content generally was higher than in non-inoculated plants. In general, mycorrhizal inoculated plant P % content was over critical levels (Table 3). The plants Zn content was varied between AMF species. In seed stages, cocktail mycorrhizae inoculation resulted in 20.8 mg Zn kg^{-1} SDW. However *G. mosseae* inoculated plants resulted in less Zn content 14.8 mg Zn kg^{-1} SDW (Table 3). In seedling stages *G. intraradices* inoculated plants had higher Zn content (23.7 mg Zn kg^{-1} SDW).

Root infection was significantly affected by mycorrhizal inoculation. All AMF species resulted in more than 30 % of root infection (Table 4). There were no differences between seed and seedling inoculation.

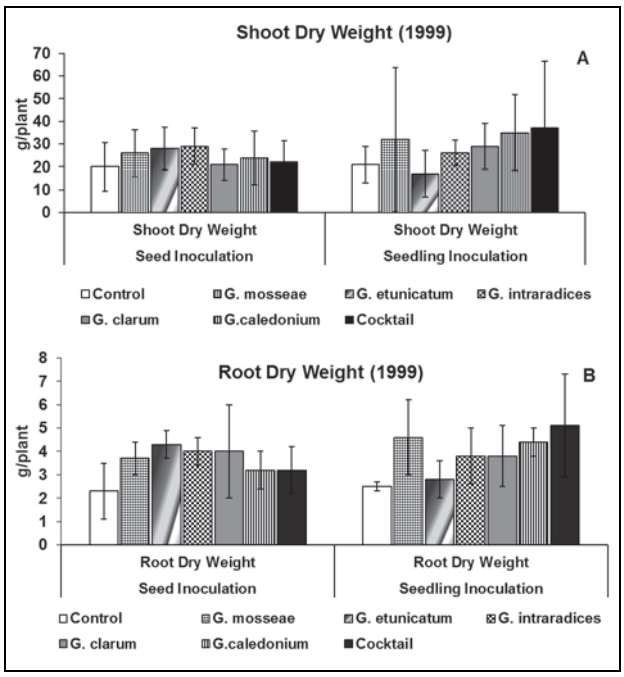


Fig. 1. Effect of mycorrhizal species and inoculation time (seed and seedling stages) on shoot and root dry weight in experiment I (year 1999).

Experiment II (Year 2000)

In 2000, plants were harvested twice and it was found that *Glomus clarum* was the most effective AMF species. In the first harvest in seed inoculation, non-inoculated plants produced 3.5 g plant^{-1} dry weight (SDW) while *G. mosseae* inoculated plants produced 12.0 g plant^{-1} SDW (Fig. 2). In seedling inoculation control, plants produced 7.6 g plant^{-1} SDW whereas *G. etunicatum* inoculated plants produced 10.5 g plant^{-1} SDW. In the second harvest, there were fewer differences between inoculated and non-inoculated plants. For example, non-inoculated plants produced 14.1 g plant^{-1} SDW while *G. clarum* inoculated plants produced 19.6 g plant^{-1} DW. In seedling stages non-inoculated plants produced 16.7 g plant^{-1} DW but *G. clarum* inoculated plants produced 27.3 g plant^{-1} SDW. This is also statistically significant $P < 0.003$ (Table 1). *G. clarum*, *G. etunicatum* and cocktail were also the effective AMF species. Plant root growth was similar to shoot SDW results. Except cocktail mycorrhiza inoculated plants had higher RDW.

Flowering time between AMF species was a few days earlier (Table 2). Compared to control plants, *G. etunicatum* inoculated tomato plants flowered 6 days early.

P content increased with mycorrhizal inoculation in the first harvest, mycorrhizal inoculated plant % P content was higher than non-inoculated plants. In the second

Table 1. Significance of P-values (probability) from analysis of variance for the parameters shoot and root dryweight (DW), phosphorus (P) and zinc (Zn) concentration and root colonization for experiments (years 1999, 2000, 2001).

Years	Treatment	DF	Shoot DW	Root DW	P (%)	Zn (mg kg^{-1})	Colonization (%)
1999	Application Time (T)	1	0.595	0.314	0.835	0.052	0.474
	Mycorrhiza Species (M)	6	0.046	0.014	0.023	0.049	0.001
	T × M	6	0.198	0.048	0.253	0.130	0.032
2000	Harvest (H)	1	0.001	0.001	0.027	0.001	0.390
	Application Time (T)	1	0.001	0.366	0.001	0.142	0.892
	Mycorrhiza Species (M)	6	0.001	0.001	0.003	0.023	0.001
	H × T	1	0.098	0.491	0.223	0.288	0.289
	H × M	6	0.099	0.001	0.646	0.337	0.790
	T × M	6	0.238	0.382	0.039	0.682	0.297
	H × T × M	6	0.216	0.094	0.326	0.865	0.957
2001	Harvest (H)	1	0.458	0.173	0.567	0.981	0.001
	Application Time (T)	1	0.120	0.582	0.866	0.001	0.748
	Mycorrhiza Species (M)	6	0.001	0.001	0.376	0.014	0.001
	H × T	1	0.217	0.451	0.711	0.009	0.959
	H × M	6	0.013	0.049	0.968	0.676	0.166
	T × M	6	0.271	0.385	0.999	0.156	0.060
	H × T × M	6	0.391	0.032	0.999	0.850	0.509

DF: Degree of freedom

harvest, mycorrhizal inoculation significantly increased plant P content such as in non-inoculated plant 0.15 % but in *G. clarum* and cocktail inoculated plant it was 0.35 % P (Table 3). Mycorrhizal inoculation in both harvests increased plant Zn content. Since the Zn content was over the critical level, it makes more sense (Table 3).

Plant roots were significantly infected by mycorrhizal inoculation. In the first harvest there were no infections in non-inoculated control plants but in the second harvest there was a small contamination in non-inoculated plants (Table 4). In the first harvest *G. clarum* was the only inoculum that resulted in a higher root infection ratio.

Experiment III (Year 2001)

In 2001, mycorrhiza inoculated tomato plants grew better than non-inoculated ones. In the first harvest in seed inoculation non-inoculated tomato plants produced 1.10 g plant^{-1} (SDW) but *G. caledonium* inoculated plants produced 5.30 g plant^{-1} SDW. In seedling stages inoculation control plants produced 1.80 g plant^{-1} SDW but *G. mosseae* inoculated plants produced 5.20 g plant^{-1} SDW (Fig. 3). In the second harvest also, inoculated plants had higher total dry weight than non-inoculated plants. In particular, *G. caledonium* was the only species that produced higher shoot dry weight for both inoculation times. For both harvests mycorrhizal inoculation statistically made a significant SDW increase (Table 1). Plant root DW also increased with mycorrhizal inoculation for both inoculation and harvest times. In generally *G. caledonium* inoculated plants have high RDW.

Mycorrhizal inoculated tomato plants also flowered one week earlier in both inoculation times. Especially in seedling stages there were 8 days between non-inoculated plants (Table 2).

Plant P content was generally higher for mycorrhiza inoculated plants compared to non inoculated one. Although mycorrhizal inoculation increased plant % P content, this difference was not statistically significant (Table 3).

Plant Zn content was analyzed and in the first harvest at seed inoculation, control plants have 22.7 mg Zn kg^{-1} SDW but cocktail mycorrhizae inoculated plants have 29.2 mg Zn kg^{-1} SDW (Table 3). But these differences are statistically not significant. A similar situation was observed for seedling stages inoculation. But in the second harvest, the effect of mycorrhizal inoculation on Zn content was found to be statistically significant. Mycorrhizal inoculation significantly affected plant root colonization. In non-inoculated plants, it was 5.1–8.6 %. Also there were great differences between AMF species. The differences were between 44.7 and 62.0 % in both inoculation time and harvest time (Table 4).

Table 2. Effect of mycorrhizal species and inoculation time (seed and seedling stages) on tomato flowering time in experiment I (1999), II (2000) and III (2001).

Years	Mycorrhizal Species	Seed inoculation		Seedling inoculation	
		Flowering time	EFCC*	Flowering time	EFCC
1999	Control	18 June 1999		20 June 1999	
	G. mosseae	08 June 1999	10	08 June 1999	12
	G. etunicatum	12 June 1999	6	14 June 1999	6
	G. intraradices	08 June 1999	10	14 June 1999	6
	G. clarum	12 June 1999	6	08 June 1999	12
	G. caledonium	08 June 1999	10	05 June 1999	15
	Cocktail	10 June 1999	8	08 June 1999	12
2000	Control	29 May 2000		29 June 2000	
	G. mosseae	27 May 2000	2	26 June 2000	3
	G. etunicatum	23 May 2000	6	26 June 2000	3
	G. intraradices	26 May 2000	3	26 June 2000	3
	G. clarum	27 May 2000	2	27 June 2000	2
	G. caledonium	27 May 2000	2	27 June 2000	2
	Cocktail	27 May 2000	2	28 June 2000	1
2001	Control	07 June 2001		09 June 2001	
	G. mosseae	05 June 2001	2	04 June 2001	5
	G. etunicatum	02 June 2001	5	01 June 2001	8
	G. intraradices	03June 2001	4	03 June 2001	6
	G. clarum	02 June 2001	5	03 June 2001	6
	G. caledonium	02 June 2001	5	03 June 2001	6
	Cocktail	02 June 2001	5	01 June 2001	8

* EFCC: Early flowering days compared to control treatment
Mean of six replicates

Discussion

The results show that tomato plants inoculated with selected mycorrhiza fungal species showed better growth performance than non-inoculated plants in three years of experiments. In seed and seedling inoculation stages, compared to non-inoculated plant mycorrhizae, plant growth and nutrient uptake significantly increased. However there were no significant differences between AMF species for both inoculation times. Tomato plants, *G. clarum*, *G. mosseea*, *G. etunicatum* and cocktail were the significant mycorrhizal inoculation. Although there were no specific species for both inoculation times and three years repetition, *G. clarum* was the one that gave the higher response for three years. In seed and seedling stages, *G. mosseae* and *G. Clarum* inoculated plant have high MD compared to other species. This mean both mycorrhizal species can be suggested for seedling production. Also (Wen-Ying and Xian-Gui 1989) tested several plants' mycorrhizal dependency and inoculation significantly contributed to the plant growth. During 1999–2001, tomato plants gave a higher response to mycorrhizal inoculation in similar field experiments performed (Ortas 2008, 2012). It was also found that plants flowered earlier and had an earlier yield. Ortas (2010) observed, under field conditions mycorrhizal inoculation significantly increased plant growth and this increase was not only directly related to the plant nutrient uptake performance but also related to the early root growth and adaptation to the soil conditions.

Since mycorrhizal inocula were propagated naturally each year, the quality and quantity of spores were different. Although it has been speculated that plant species depends on specific AMF species, there were several factors affecting the efficiency of mycorrhizal inoculation such as the number of spores per plant, inoculum quality, plant species and inoculum time and growth media conditions. The literature on mycorrhizal inoculation with seedling is controversial (Saggin-Junior and De Silva 2006). Similar experiments were conducted for pepper and eggplant with the same mycorrhizal species by (Ortas et al. 2011a, b). They reported that there is no specific mychorriza have the same effect for three years experiment.

Table 3. Effect of mycorrhizal species and inoculation time (seed and seedling stages) on tomato P (%) and Zn (mg Zn kg^{-1} DW) concentration.

Years	Harvests	Inoculation methods	Mycorrhizal Species						
			Control	*G. mosseae*	*G. etunicatum*	*G. intraradices*	*G. clarum*	*G. caledonium*	Cocktail
P concentration (%)									
1999		#Seed	0.20 ± 0.1 c	0.18 ± 0.0 c	0.27 ± 0.1 b	0.26 ± 0.0 b	0.27 ± 0.1 b	0.24 ± 0.0 bc	0.23 ± 0.1 bc
		Seedling	0.21 ± 0.0 bc	0.22 ± 0.1 bc	0.31 ± 0.0 a	0.21 ± 0.0 bc	0.23 ± 0.1 bc	0.28 ± 0.1 b	0.23 ± 0.0 bc
2000	1th	Seed	0.24 ± 0.0 abc	0.26 ± 0.1 abc	0.33 ± 0.1 a	0.28 ± 0.0 abc	0.31 ± 0.1 ab	0.31 ± 0.1 ab	0.32 ± 0.0 ab
		Seedling	0.18 ± 0.1 c	0.24 ± 0.1 abc	0.20 ± 0.0 bc	0.20 ± 0.1 bc	0.22 ± 0.0 abc	0.20 ± 0.0 bc	0.22 ± 0.0 abc
	2nd	Seed	0.15 ± 0.0 cd	0.21 ± 0.1 bcd	0.27 ± 0.1 ab	0.21 ± 0.1 bcd	0.35 ± 0.0 a	0.20 ± 0.0 bcd	0.35 ± 0.0 a
		Seedling	0.13 ± 0.1 d	0.24 ± 0.1 bc	0.22 ± 0.1 bcd	0.23 ± 0.1 bcd	0.17 ± 0.0 cd	0.19 ± 0.0 bcd	0.19 ± 0.0 bcd
2001	1th	Seed	0.19 ± 0.0 a	0.21 ± 0.0 a	0.22 ± 0.0 a	0.23 ± 0.0 a	0.21 ± 0.0 a	0.23 ± 0.0 a	0.21 ± 0.0 a
		Seedling	0.18 ± 0.0 a	0.21 ± 0.0 a	0.21 ± 0.0 a	0.21 ± 0.0 a	0.21 ± 0.0 a	0.20 ± 0.0 a	0.19 ± 0.0 a
	2nd	Seed	0.21 ± 0.0 a	0.22 ± 0.0 a	0.23 ± 0.0 a	0.23 ± 0.0 a	0.23 ± 0.0 a	0.24 ± 0.0 a	0.23 ± 0.0 a
		Seedling	0.19 ± 0.0 a	0.21 ± 0.0 a	0.22 ± 0.1 a	0.22 ± 0.0 a	0.23 ± 0.0 a	0.23 ± 0.0 a	0.23 ± 0.0 a
Zn concentration (mg Zn kg^{-1} DW)									
1999		Seed	18.5 ± 3.1 ab	14.8 ± 1.7 b	16.9 ± 3.4 b	18.9 ± 7.4 ab	17.8 ± 4.4 ab	18.2 ± 4.5 ab	20.8 ± 3.9 ab
		Seedling	21.0 ± 1.0 ab	23.2 ± 1.4 a	21.2 ± 2.0 ab	23.7 ± 1.2 a	19.5 ± 8.8 ab	21.6 ± 5.4 ab	18.1 ± 5.0 ab
2000	1th	Seed	33.4 ± 5.1 ab	42.9 ± 11.4 a	41.1 ± 19.1 a	43.4 ± 4.9 a	41.5 ± 3.4 a	38.7 ± 6.6 a	35.1 ± 2.8 ab
		Seedling	24.3 ± 2.7 b	37.2 ± 1.4 ab	41.0 ± 4.1 a	34.7 ± 3.8 ab	39.6 ± 2.2 a	37.4 ± 5.7 ab	34.7 ± 4.7 ab
	2nd	Seed	25.5 ± 3.9 a	35.4 ± 8.6 a	28.0 ± 6.8 a	37.4 ± 12.0 a	27.7 ± 4.9 a	38.8 ± 14.2 a	33.7 ± 2.0 a
		Seedling	27.7 ± 1.7 a	32.6 ± 9.3 a	34.0 ± 2.9 a	32.9 ± 0.7 a	28.5 ± 0.8 a	31.6 ± 6.7 a	34.9 ± 2.5 a
2001	1th	Seed	22.7 ± 3.7 a	26.6 ± 2.5 a	27.1 ± 3.2 a	27.0 ± 1.6 a	28.9 ± 4.3 a	25.5 ± 7.0 a	29.2 ± 8.2 a
		Seedling	21.2 ± 1.9 a	35.9 ± 16.8 a	34.1 ± 4.8 a	36.4 ± 4.1 a	30.9 ± 4.3 a	36.3 ± 6.5 a	36.1 ± 2.1 a
	2nd	Seed	18.7 ± 1.6 c	23.8 ± 1.5 bc	24.0 ± 3.6 bc	24.8 ± 0.7 bc	25.4 ± 2.3 bc	25.1 ± 3.5 bc	25.7 ± 5.7 bc
		Seedling	25.8 ± 5.9 bc	46.3 ± 6.1 a	40.0 ± 7.9 ab	31.9 ± 5.4 abc	33.3 ± 12.2abc	40.5 ± 13.4ab	39.3 ± 4.8 ab

Mean of six replicates ± SD. Means in the same row followed by the same letter represent significant differences ($P \leq 0.05$) among treatments.

comparison of means of Duncan's were calculated for each year separately

It has been demonstrated AM fungi inoculated seedlings exhibited better transplant performance due to it's higher shoot fresh weight (avg. 11.28 g $plant^{-1}$), high shoot/root ratio (avg. 0.236), higher root biomass (avg. 2.17 g $plant^{-1}$) (Oseni et al. 2010). Also Ren et al. (2010) and Nzanza et al. (2011) observed that the AM colonization increased root growth of tomato. Franco et al. (2011) reported that AMF can also induce changes in the root system of the host plant that may improve it's resistance to several abiotic stresses. Also the percentages of mycorrhizal colonization values were relatively low. In our previous experiment, plant root colonization data were low (Ortas et al. 2011a, b).

Through three years of experiments, mycorrhizal inoculated tomato plants were taller than non-inoculated plants, but plant diameter did not vary between inoculated and non-inoculated plants (unpublished data). Karagiannidis et al. (2002b) and Ortas et al. (2011a) reported that inoculation with the AMF significantly increased dry shoot weight in tomato plants, compared to the control plants. There was a favorable effect on mycorrhizal inoculated tomato plants which flowered as early as 5–10 days from year to year. In experiment II (the year 2000), there were no differences for early flowering between inoculated and non-inoculated plants. Although the reason is not known, flowering time may be affected indirectly by varying inoculum quality from year to year. It is very important to produce early tomato fruits for market in cost of Mediterranean countries.

Ortas and Akpinar (2006) and Ortas et al. (2011b) observed that mycorrhizae inoculated pepper and eggplant plants have early flowering than non inoculated plants.

Table 4. Effect of mycorrhizal species and inoculation time (seed and seedling stages) on tomato root infection (%).

Years	Harvests	Inoculation methods	Mycorrhizal Species						
			Control	*G. mosseae*	*G. etunicatum*	*G. intraradices*	*G. clarum*	*G. caledonium*	Cocktail
Root colonization (%)									
1999		#Seed	11 ± 3 c	44 ± a	36 ± 11 ab	39 ± a	40 ± 6 a	33 ± 5 ab	39 ± 9 a
		Seedling	13 ± 2 c	29 ± 13 b	47 ± 3 a	36 ± 4 b	33 ± 9 ab	36 ± x ab	36 ± 15ab
2000	1th	Seed	0 ± 0 d	20 ± 3 c	35 ± 2 ab	21 ± 14 c	41 ± 5 a	25 ± 7 bc	32 ± 5 abc
		Seedling	4 ± 4 d	31 ± 9 abc	27 ± 6 bc	29 ± 13 abc	42 ± 7 a	32 ± 5 abc	35 ± 7 a
	2nd	Seed	11 ± 5 bc	26 ± 7 abc	41 ± 20 ab	26 ± 14 abc	45 ± 28 ab	24 ± 4 abc	39 ± 7 ab
		Seedling	4 ± 1 c	41 ± 28 ab	21 ± 3 ac	26 ± 8 abc	33 ± 17 abc	22 ± 3 ac	47 ± 39 a
2001	1th	Seed	8.6 ± 5.3 b	48.0 ± 3.6 a	44.7 ± 4.5 a	52.3 ± 6.7 a	5.7 ± 8.1 a	54.7 ± 1.2 a	46.7 ± 7 a
		Seedling	8.2 ± 8.2 b	44.7 ± 3.2	47.0 ± 2.6 a	51.0 ± 11 a	47.7 ± 6.7 a	54.3 ± 9.3 a	58.0 ± 1.7 a
	2nd	Seed	7.6 ± 6.3 c	59.0 ± 5.3 a	45.3 ± 1.2 ab	62.0 ± 2.0 a	59.7 ± 5.1 a	55.0 ± 5.3 ab	55.0 ± 2.6 ab
		Seedling	5.1 ± 4.5 b	49.0 ± 7.5 a	59.7 ± 6.1 a	60.0 ± 6.0 a	60.7 ± 4.9 a	53.3 ± 3.2 ab	58.6 ± 16.3 a

Mean of six replicates ± SD. Means in the same row followed by the same letter represent significant differences ($P \leq 0.05$) among treatments.

comparison of means of Duncan's was calculated for each year separately

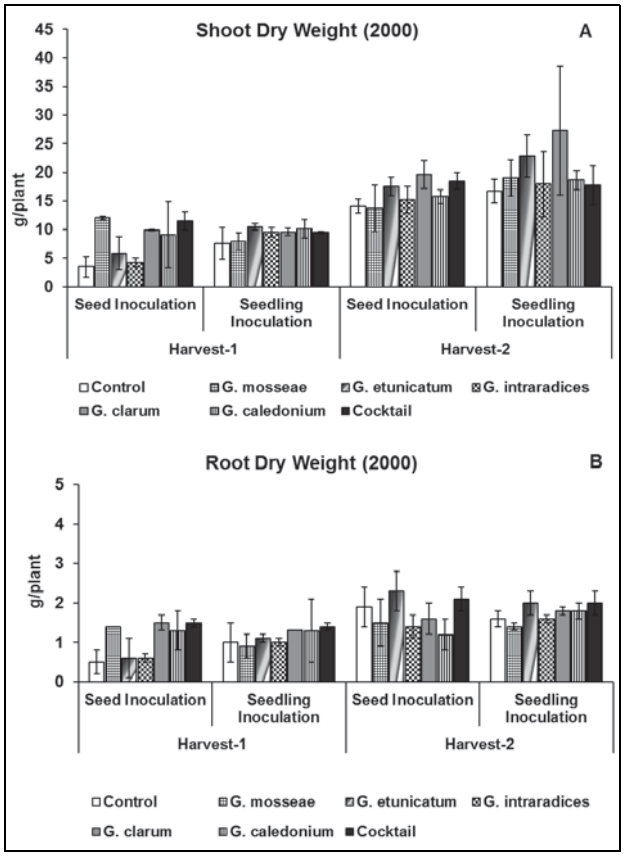


Fig. 2. Effect of mycorrhizal species and inoculation time (seed and seedling stages) on shoot and root dry weight in experiment II (year 2000).

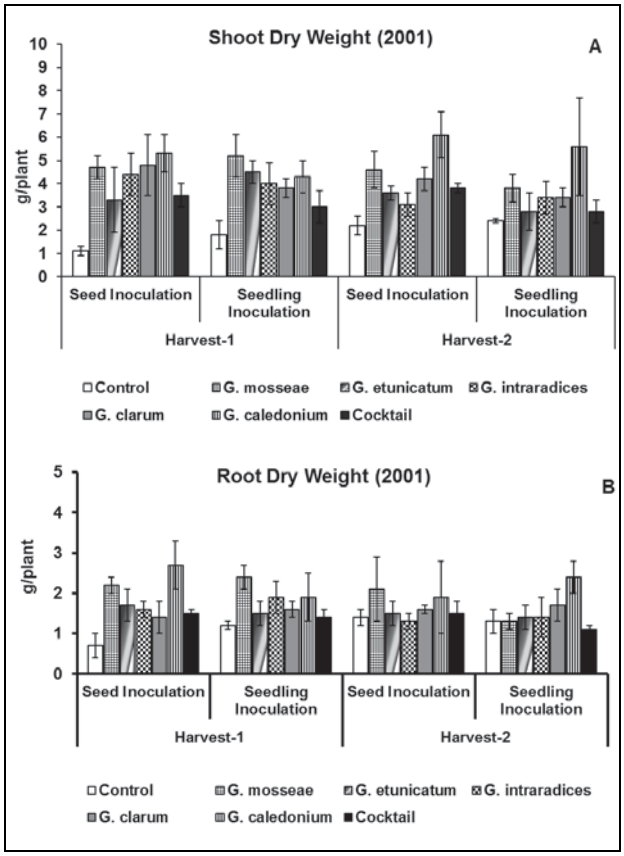


Fig. 3. Effect of mycorrhizal species and inoculation time (seed and seedling stages) on shoot and root dry weight in experiment III (year 2001).

Especially for early spring time, even one week of early production is very profitable for producers. Scagel (2004) reported that, the rhizosphere organisms associated with the AMF inoculum influenced several measures of plant development, growth and flower production of harlequin flower. Similarly (Sohn et al. 2003) found that AMF-inoculation significantly shortened flowering time compared to non-AMF plants. There are very few studies of hormonal regulation of AM formation on root colonization and plant development, but not on plant flowering shortened.

Experiments performed in seed sowing and transplanting stages showed that the survival rate was higher in inoculated plants. Menge et al. (1978) reported that mycorrhizal inoculated plants were more successful at transplanting stage against early shock. Since mycorrhizae make plant roots stronger, inoculated seedling roots are more successful in recovery and uptake nutrient and water.

AMF significantly increased total P content of seedlings. In general mycorrhizal inoculated seedling P % content was over critical levels. It seems that growth medium P_2O_5 content significantly increased plant P % content. (Sylvia and Chellemi 2001; Latef and He 2011) conclude that reduced P application may allow tomatoes to take advantage of their inherent responsiveness to mycorrhizae in a low to moderate soil-P environment.

Mycorrhizal inoculation significantly increased plant Zn content ($P < 0.05$). Non-inoculated plants had 20 mg Zn kg^{-1} SDW but inoculated plants had nearly 40 mg Zn kg^{-1} SDW. An agreement with previous, mycorrhizal inoculation increases Zn uptake of plants (Kothari et al. 1991; Marschner 1998; Liu et al. 2000; Ortas 2003, 2010; Ortas et al. 2011a; Watts-Williams and Cavagnaro 2012). Since plant critical Zn content was 20 mg Zn kg^{-1} SDW (Jones 1998), the results obtained in seedling stages are very important for plant Zn nutrition. Since the experiment soils are Zn deficient (Ortas 2008) mycorrhizal inoculation is very important. The mycorrhizal response to nutrient uptake and plant growth is generally related to the size of the nutrient depletion zone that develops around a root (O'Keefe and Sylvia 1991; Ortas 1996). Cimen et al. (2010) observed that mycorrhiza inoculated tomato plants P, K, Mg, Fe, Mn, Zn and Cu in leaves were higher in non-inoculated. It has been reported that mycorrhizal colonization increased P (Dennett et al. 2011) and Zn (Cavagnaro et al. 2006, 2008) content of tomato plants. Although this plant is not highly responsive to AM fungi in terms of plant growth (Smith et al. 2009), however there is significant effect on plant growth nutrient uptake. For example, the Zn content in shoots and fruits of field-grown wild-type mycorrhizal tomato plants was found to be up to 50 % higher than in a mutant with reduced mycorrhizal colonization (Cavagnaro et al. 2006).

The successful application of mycorrhizal inoculum for tomato seedling production requires improved strategies to target field transplantation. Still there is some difficulty in extrapolating data from pot experiments to field applications. Our previous work has shown that in low-P soil fertility conditions, tomato plants have given a higher response to mycorrhizal inoculation (Ortas 2012).

Conclusions

The results demonstrated that inoculation with AMF species had positive effects on seedling growth of tomatoes. In both inoculation treatments (seed and seedling stage), although the positive effect was not as profound as in seedling inoculation, treatment with AMF species had advantages on seeds as well. Seed stage inoculation has higher mycorrhizal dependency than seedling stage inoculation. Mycorrhizal inoculated plants flowered earlier than non-inoculated plants. Plants responses to the mycorrhizal inoculation were different for both inoculation times. There were no specific AMF species for both inoculation times and three years repetition but *G. clarum* inoculation seeds to be one of efficient inoculum. In general, cocktail *G. clarum*, *G. mosseae*, *G. caledonium*, *G. intraradices* and *G. etunicatum* significantly colonized plant roots. Mycorrhizal inoculation also increased plant P and Zn content. The future research should be directed to continue under field conditions with different inoculum ratio.

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