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Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions

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Abstract

Mycorrhiza application in horticultural production in the Eastern Mediterranean region of Turkey has been studied under field conditions for several years. The effects of different arbuscular mycorrhizal fungi (AMF) have been evaluated under field conditions for cucumber production. The parameters measured were seedling survival, plant growth and yield, and root colonization. In 1998 and 1999, *Glomus mosseae* and *Glomus etunicatum* inoculated cucumber seedlings were treated with and without P (100 kg P_2O_5 ha^{-1}) application. A second experiment was set up to evaluate the response of cucumber to the inoculation with a consortia of indigenous mycorrhizae*, G. mosseae, G. etunicatum, Glomus clarum, Glomus caledonium* and a mixture of these four species. Inoculated and control non inoculated cucumber seedlings were established under field conditions in 1998, 2001, 2002 and 2004. Seedling quality, seedling survival under field conditions and yield response to mycorrhiza were tested. Fruits were harvested periodically; at blossom, plant leaves and root samples were taken for nutrient content and mycorrhizal colonization analysis respectively. The field experiment results showed that mycorrhiza inoculation significantly increased cucumber seedling survival, fruit yield, P and Zn shoot concentrations. Indigenous mycorrhiza inoculum was successful in colonizing plant roots and resulted in better plant growth and yield. The relative effectiveness of each of the inocula tested was not consistent in the different experiments, although inoculated plants always grew better than control no inoculated. The most relevant result for growers was the increased survival of seedlings.

Additional key words: *Cucumis sativus* L., horticulture, mycorrhizal species, plant nutrition.

Resumen

Efecto de la aplicación de micorrizas sobre el crecimiento vegetal y la absorción de nutrientes en la producción de pepino bajo condiciones de campo

Se han evaluado los efectos de diferentes hongos formadores de micorrizas arbusculares (HMA) en la producción de pepino en la zona del Mediterráneo Oriental de Turquía. Los parámetros medidos fueron supervivencia de plántulas, crecimiento de la planta, su rendimiento en frutos y la colonización micorrícica de la raíz. En 1998 y 1999 se inocularon plántulas de pepino con *Glomus mosseae* y *Glomus etunicatum* y se realizó un tratamiento control y un tratamiento fertilizado con P (100 kg P_2O_5 ha^{-1}). En un segundo experimento en campo se evaluó la respuesta del pepino a la inoculación con distintos HMA nativos (*G. mosseae*, *G. etunicatum*, *Glomus clarum*, *Glomus caledonium* y una mezcla de estas cuatro especies). En 1998, 2001, 2002 y 2004 se plantaron en campo plántulas de pepino, control (no inoculadas) e inoculadas. Se evaluó la calidad de las plántulas, su supervivencia y su rendimiento en frutos. Se cosecharon los frutos periódicamente y, en el momento de la floración, se tomaron muestras de hojas y raíz para determinar el contenido de nutrientes y la micorrización, respectivamente. La inoculación de HMA aumentó significativamente la supervivencia de plántulas de pepino, la producción de frutos, y las concentraciones foliares de P y Zn; también fue efectivo en el establecimiento de la simbiosis en raíz y dio como resultado un mayor crecimiento de las plantas y un aumento de la cosecha. La eficacia relativa de cada uno de los inóculos probados no fue consistente, ya que varió según el año del ensayo, aunque las plantas micorrizadas siempre crecieron mejor que las plantas no inoculadas. El resultado más relevante para los productores fue el aumento de la supervivencia de las plántulas.

Palabras clave adicionales: *Cucumis sativus* L., especificidad de inóculo, horticultura, nutrición de plantas.

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Abbreviations used: AMF (arbuscular mycorrhizal fungi).

Introduction

Horticultural cultivation is becoming widespread in the Mediterranean region of Turkey. Soils in the Çukurova region have high levels of clay and lime that cause P, Zn, Fe and Mn deficiency. Increased use of fertilizers to correct these deficiencies can affect both human health and the environment. A reduction of the fertilizers input together with selecting cultivars and species better adapted to these constrains is necessary to increase the sustainability of crop production.

Arbuscular mycorrhizal fungi (AMF) can be integrated in soil management to achieve low-cost sustainable agricultural systems (Hooker and Black, 1995). Mycorrhizal fungi occur in most of the soils and colonize roots of many plant species. Mycorrhiza are the structures resulting from the symbiosis between these fungi and plant roots, and are directly involved in plant mineral nutrition. The symbiotic root-fungal association increases the uptake of less mobile nutrients (Ortas *et al.*, 2001), essentially phosphorus (P) but also of micronutrients like zinc (Zn) and copper (Cu), the symbiosis has also been reported as influencing water uptake. AMF can also benefit plants by stimulating the production of growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses and increasing resistance to pests and soil borne diseases (Al-Karaki, 2006). These benefits are mainly attributed to improved phosphorous nutrition (Plenchette *et al.,* 2005). Most horticultural and crop plants establish the symbiosis with AMF. Although the mycorrhizal potential of the symbiosis to improve horticultural production is recognized (Estaun *et al*., 2002), it has not been implemented under field conditions, integrating this biotechnology in large horticultural production systems (Ortas, 2008).

Cucumber (*Cucumis sativus* L.), a common vegetable in the Mediterranean region, forms the arbuscular mycorrhizal symbiosis (Kubota *et al*., 2005), with a positive effect on plant growth (Ortas, 2008) under controlled experimental conditions. Cucumber crop responds well to an increased fertilization; Sainz *et al*. (1998) reported that the amendment of soil with vermicompost in quantities ranging from 10 to 50% significantly increased dry matter yields of cucumber plants, compared to treatments where soil was not amended. Cagras *et al*. (2000) used *G. mosseae* and *G. fasciculatum* mycorrhizae spores to inoculate cucumber plants under sterile and non-sterile conditions in Çukurova region (Turkey) and found that inoculated plants had a higher uptake of P, Zn and Mn. Wang *et al*. (2008) also inoculated cucumber seedlings with three AMF, and found an increase in the concentrations of nitrogen (N) and P in roots and magnesium (Mg), Cu, and Zn concentration in shoots. Cucumber production starts with seedling production in the nursery, the seedlings that are then transplanted to the field. Mycorrhizal inoculation of cucumber could increase seedling quality and reduce the quantity of fertilizer application, to achieve similar yields to non-inoculated plants (Charron *et al*., 2001; Ortas, 2003, 2008).

In this work we attempted to find a niche for the production of mycorrhizal inoculated seedling with the objective of substituting partially or completely the heavy fertilizer application with mycorrhiza inoculation. We screened different mycorrhizal species assessing the effects on cucumber seedling survival, growth, nutrient uptake and yield under field conditions.

Material and methods

Field experiments were carried out on an Arik clay-loam soil, which was classified as an Entic Chromoxerert in the Agricultural Experimental Station of Çukurova University, Adana, in southern Turkey (Research farm is located between 37° 01’ 08”-37° 01’18” North Latitude and 35° 21’ 45”/-35° 21’ 33” East Longitude). Soil chemical and biological properties are presented in Table 1.

Seedling production

Nursery trial experiments were carried out on the Çukurova University Research Farm, Adana, Turkey.

Table 1. Agricultural Experimental Station (Çukurova University) soil characteristics

	Depth 0-20 cm	**Depth 20-40 cm**
Organic matter(%)	1.10	0.80
$CaCO_3$ (%)	30.4	33.1
pH (H_2O)	7.8	7.8
P (mg kg^{-1})	3.65	4.35
Fe (mg kg^{-1})	5.43	5.66
Mn (mg kg^{-1})	5.74	5.31
Zn (mg kg^{-1})	0.52	0.23
Cu (mg kg^{-1})	1.86	1.56
No. AMF spores/10 g soil	36	17

AMF: arbuscular mycorrhizal fungi.

Mycorrhizal and non-mycorrhizal cucumber seedlings were produced from seeds under glasshouse conditions. Cucumber seeds (Yayla F1 local variety) were sown in a sand-soil-compost (7:2:1 v/v) growth medium for seedling production. The substrate was autoclaved at 121°C for 2 h prior to use as a growth media. Seeds were sown in trays. Mycorrhiza spores were added to each respective mycorrhizal treatment, non-mycorrhizal plants received mycorrhiza spore-free medium.

Field experiments

The experiments were carried out during 1998-2004 in greenhouse and field conditions. Two experiments were conducted. In the first experiment during 1998 and 1999 there were three inoculation treatments, control non inoculated, *G. mosseae* and *G. etunicatum* inoculated cucumber seedlings and two P amendment treatments: no P and 100 kg P_2O_5 ha^{-1} . In the second experiment there were seven mycorrhiza treatments: control non inoculated seedlings, seedlings inoculated with indigenous mycorrhiza (collected from a research farm and re-cultured on maize host plant) *G. mosseae, G. etunicatum, G. clarum, G. caledonium* and a mixture of these species. Before transplanting to the field, mycorrhizal seedlings were re-inoculated: 1,000 spores per seedling were applied to the field soil, below the seedling roots. The plots were 3 × 5 m (15 m^2) and there were three replicated experimental plots per treatment. The experiment was repeated in 1998, 2001, 2002 and 2004. Seedling survival after transplant, under field conditions, plant yield and nutrient uptake were determined. Cucumber fruits were collected throughout the season and plant leaves and root samples were taken for nutrient content and mycorrhizal colonization measurement in the flowering period. Mycorrhizal colonisation assessment was done after staining the root samples (Koske and Gemma, 1989) using a grid-line intersect method (Giovannetti and Mosse, 1980). Plant tissue analysis was done using adult leaves collected at the flowering period. Leaves were dried at 75°C, ground with a Tema mill and digested with HCl. P concentration was measured colorimetrically (Murphy and Riley, 1962) and Zn was measured with a spectrophotometer.

Data obtained were analyzed using analysis of variance procedures (SAS Inst., 1989) and treatment means were compared using a LSD test.

Results

First experiment

Before transplanting, at the end of the seedling stage AMF root colonization was monitored. Inoculated plants presented the symbiosis. Cucumber seedlings inoculated with *G. mosseae* or *G. etunicatum* or non-inoculated were transplanted to the field. The seedling survival was evaluated and AMF inoculated seedlings presented a higher survival than non inoculated plants, despite the P amendment (Table 2).

In 1998 and 1999, *G. mosseae* and *G. etunicatum* inoculated seedlings were transplanted to field conditions with and without phosphorus treatment. Mycorrhizal inoculation increased the cucumber fruit yield compared with non-inoculated plants for both fungi used (Fig. 1). The increased P supply did not increase yield significantly

In the 1998 experiment, there were significant differences between mycorrhizal and non mycorrhizal treatments in the P and Zn content; however, in 1999, the differences between mycorrhiza and non mycorrhiza plants were no longer significant.

Mycorrhizal root colonization was determined at the flowering stage and control, non inoculated seedlings were found to have established the symbiosis in the field, achieving up to 27% root colonization although

Table 2. Effect of mycorrhizal inoculation on cucumber seedling mortality after transplanting to the field under –P/+P treatments

Plant species	–P				+P		
	Seedlings in the plot (No.)	Dead seedlings (No.)	Dead seedlings (%)	Surviving seedlings (%)	Dead seedlings (No.)	Dead seedlings (%)	Surviving seedlings (%)
Control	24	14	58	**42**	17	71	**29**
G. mosseae	24	2	10	**90**	4	15	**85**
G. etunicatum	24	5	19	**81**	6	24	**76**

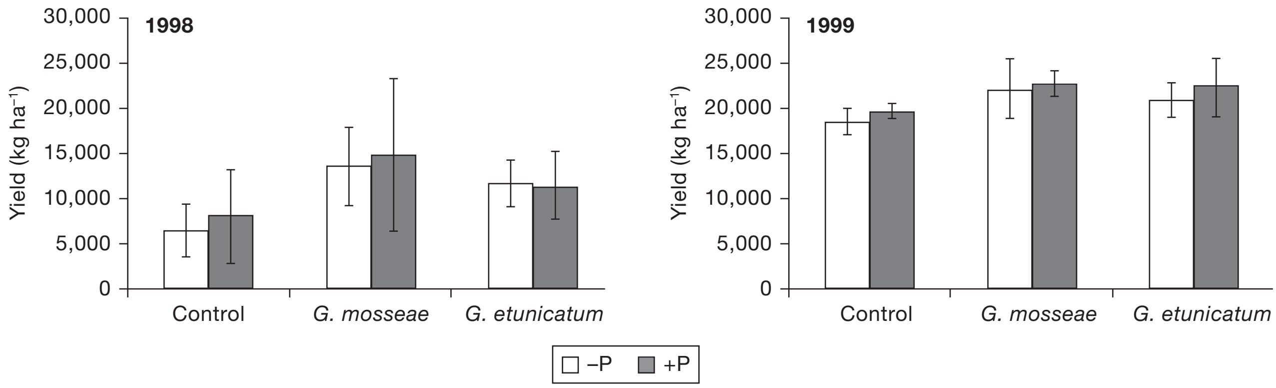

Figure 1. Effect of P leel and mycorrhizal inoculation on cucumber yield under field conditions.

mycorrhiza inoculated plants showed a higher percentage of root colonization (Table 3). Mycorrhizal inoculation increased plant P concentration, non-inoculated plants, despite the presence of the symbiosis established with native endophytes, had lower P content than inoculated plants. P amendment increased P tissue concentration in all treatments (Table 3). Cucumber Zn plant tissue concentration was also increased by mycorrhizal inoculation.

Second experiment

This second experiment was repeated for four growing seasons, in 1998, 2001, 2002 and 2004, and the results from each year are different. However, mycorrhiza inoculation always resulted in a yield increase compared to the control non inoculated plants (Fig. 2). In 1998, the best response to inoculation was shown by plants inoculated with *G. caledonium*; followed by fungal mix, the indigenous inocula and *G. mosseae* that gave a lower increase in plant yield, although it was still higher than the yield of non-inoculated plants. In 2001 the best response was found in *G. etunicatum* inoculated plants, followed by the species mix and *G. mosseae* inoculated plants that were still giving a lower increase in yield than the other fungal species assayed. In 2002, cucumber plants inoculated with the indigenous fungi performed better than the other inoculants. In the 2004 experiment, the cucumber production increased in all

Table 3. Effect of mycorrhizal species and phosphorus application on P and Zn shoot content and root colonization of cucumber plants

		P (% DW)	Zn (mg kg^{-1} DW)	Root colonization (%)
1998				
–P	Control	0.20 ± 0.01	20.97 ± 0.6	27 ± 8
	G. mosseae	0.25 ± 0.00	26.18 ± 0.4	58 ± 7
	G. etunicatum	0.27 ± 0.02	28.80 ± 1.5	61 ± 11
+P	Control	0.23 ± 0.01	21.48 ± 0.8	21 ± 5
	G. mosseae	0.27 ± 0.04	25.20 ± 2.8	66 ± 9
	G. etunicatum	0.26 ± 0.02	27.62 ± 2.2	69 ± 10
1999				
–P	Control	0.23 ± 0.04	26.60 ± 4.0	17 ± 6
	G. mosseae	0.25 ± 0.12	28.68 ± 2.4	45 ± 4
	G. etunicatum	0.29 ± 0.07	28.30 ± 2.1	49 ± 5
+P	Control	0.29 ± 0.06	24.80 ± 4.9	21 ± 6
	G. mosseae	0.30 ± 0.06	24.87 ± 5.5	46 ± 5
	G. etunicatum	0.29 ± 0.07	27.45 ± 1.1	55 ± 3

Data are means of three samples ± standard deviation.

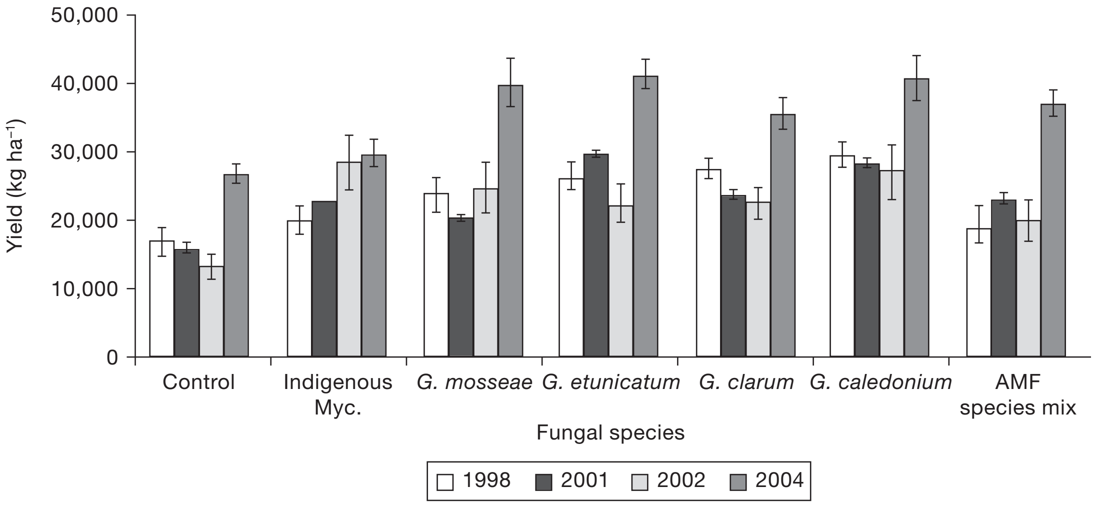

Figure 2. Effect of several mycorrhizal species inoculation on cucumber yield under field conditions.

treatments compared to the previous seasons yields, the best response to inoculation was found with *G. mosseae*, *G. etunicatum* and *G. caledonium* inoculated plants (Fig. 2) and indigenous mycorrhiza inoculation did not produce significant effects in the 2004 growing season.

Each year plant yield responded differently to the inoculation with the mycorrhiza species used. Irrespective of the year control non-inoculated plants established the symbiosis, but the root colonization level was lower that the root colonization observed in the plants inoculated, independently of the fungal species used (Table 4). Mycorrhizal colonization resulted in a significant increase in P tissue concentration of cucumber plants (Table 4). Non inoculated control plants and plants inoculated with the mycorrhizal fungal mix consistently had lower levels of tissue P, whilst inoculation with the indigenous fungi significantly increased P concentration. The tissue Zn concentrations in mycorrhizal plants were higher than those in non-mycorrhizal plants. Both Zn and P concentration in plant tissues was over critical levels in all treatments (Table 4).

Discussion

In coastal Eastern Mediterranean part of Turkey, mycorrhizal studies have been conducted since 1995 in crop and in horticultural plants. Although for crop plants changes in the management of the soil-plant system might be sufficient to optimize the mycorrhiza symbiosis, in horticulture the inoculation of seedlings prior to transplant seems to be the best option (Ortas *et al*., 2004; Ortas, 2006; Ortas and Varma, 2007). Seedling inoculation economizes inoculum, which is costly to produce, and also cucumber seedlings inoculated are more resistant to transplant. In all the experiments, the non-inoculated controls established the symbiosis; however inoculation resulted in higher plant yield and better plant nutrition. Cagras *et al*. (2000) found that mycorrhizal inoculation of cucumber plants significantly increased leaf and shoot fresh and dry weight, root biomass and leaf area index. Lee and George (2005) showed that mycorrhizal hyphae of *G. mosseae* had a significant contribution in the uptake of P, Zn and Cu by inoculated cucumber plants resulting in a increased concentration of those nutrients in the plant shoots. Wang *et al*. (2008) also showed an increased N, P, Cu and Zn uptake in inoculated cucumber plants.

The results from experiment 2 underline the main problem for including mycorrhiza inoculation in field practices: after several years of controlled field experiments the effects from different isolates used were still unpredictable. The overall cucumber plant yield changed each year, although mycorrhizal inoculation was always beneficial for plant growth. The effects of each of the isolates assayed varied between the years, therefore there was no isolate that was consistently the best; however the indigenous isolates always gave good results, establishing good root colonization and increased cucumber yield, and could be integrated in cucumber production as an ecologically sound practi-

Table 4. Effect of several mycorrhizal species on P and Zn shoot content and root colonization of cucumber plants

	P (% DW)	**Zn (mg kg^{-1} DW)**	**Root colonization (%)**
1998			
Control	0.28 ± 0.1	21.85 ± 5.3	27.42 ± 8.2
Indigenous mycorrhizae	0.36 ± 0.2	26.59 ± 5.9	62.42 ± 17.9
G. mosseae	0.32 ± 0.2	24.08 ± 4.9	66.94 ± 15.8
G. etunicatum	0.34 ± 0.1	25.62 ± 6.5	62.99 ± 16.1
G. clarum	0.31 ± 0.2	22.52 ± 7.4	79.60 ± 19.1
G. caledonium	0.35 ± 0.1	28.00 ± 8.1	70.00 ± 12.6
Mix of these fungal species	0.31 ± 0.1	23.36 ± 5.9	49.68 ± 6.9
2001			
Control	0.27 ± 0.1	29.47 ± 5.1	39.36 ± 13.5
Indigenous mycorrhizae	0.35 ± 0.2	38.44 ± 6.2	62.05 ± 14.5
G. mosseae	0.31 ± 0.2	38.90 ± 4.4	59.66 ± 17.3
G. etunicatum	0.31 ± 0.1	35.17 ± 7.0	56.08 ± 18.7
G. clarum	0.31 ± 0.1	34.21 ± 6.7	63.92 ± 17.0
G. caledonium	0.29 ± 0.1	35.11 ± 6.8	74.35 ± 12.9
Mix of these fungal species	0.23 ± 0.1	30.61 ± 8.1	36.13 ± 11.5
2002			
Control	0.20 ± 0.1	19.16 ± 5.8	30.48 ± 11.3
Indigenous mycorrhizae	0.26 ± 0.1	29.52 ± 5.5	60.97 ± 19.4
G. mosseae	0.27 ± 0.1	28.92 ± 4.1	55.89 ± 14.1
G. etunicatum	0.24 ± 0.1	27.09 ± 3.9	49.11 ± 8.8
G. clarum	0.23 ± 0.1	24.49 ± 6.4	62.10 ± 11.4
G. caledonium	0.26 ± 0.1	28.06 ± 6.9	75.89 ± 10.8
Mix of these fungal species	0.21 ± 0.1	22,74 ± 5,9	47,42 ± 14,8
2004			
Control	0.22 ± 0.1	26.90 ± 5.8	45.12 ± 9.3
Indigenous mycorrhizae	0.25 ± 0.1	35.00 ± 5.5	75.49 ± 14.4
G. mosseae	0.26 ± 0.1	34.41 ± 4.1	77.18 ± 17.1
G. etunicatum	0.33 ± 0.1	35.96 ± 3.9	70.40 ± 10.8
G. clarum	0.28 ± 0.1	36.75 ± 6.4	76.45 ± 8.4
G. caledonium	0.29 ± 0.1	31.67 ± 6.9	73.79 ± 10.8
Mix of these fungal species	0.22 ± 0.1	27.05 ± 5.9	71.94 ± 14.8

Data are mean of three samples ± standard deviation.

ce. Many horticultural plants are mycorrhiza-dependent, however, in our experimental model, under field conditions with a high P application, mycorrhizal dependency is reduced.

We can conclude that cucumber plants readily establish the arbuscular mycorrhiza symbiosis with different fungal species. The efficacy of these fungal species to increase plant yield and nutrient uptake varied between the years, although inoculation always resulted in better plant growth. Seedling inoculation significantly increased cucumber survival at transplant.

The effects of other factors involved in plant growth such as soil and weather conditions, seedling production, standardization of the inocula, and crop management techniques need to be further studied in order to effectively integrate the mycorrhiza technology in horticulture production.

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