

RESPONSE OF FOUR ZUCCHINI (*Cucurbita pepo* L.) HYBRIDS TO DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI

S. Sensoy, S. Demir*, S. Tufenkci**, Ç. Erdiñç, E. Demirel*, H. Ünsal**, G. Halifeoglu, and A. Ekinçalp

Department of Horticulture, *Department of Plant Protection, **Department of Soil Science and Plant Nutrition, Agriculture Faculty, Yüzüncü Yıl University 65080 Van, TURKEY
Corresponding author e-mail: suatsensoy@yyu.edu.tr

ABSTRACT

Four zucchini hybrids (Focus F₁, Comet F₁, Natali F₁, and Ezra F₁) inoculated by three different arbuscular mycorrhizal fungi (AMF) [*Glomus intraradices* (Gi), *Glomus etinucatum* (Ge), and *Gigaspora margarita* (Gm)] in a growth chamber experiment were evaluated for seedling traits, nutrient uptake, colonization, and relative mycorrhizal dependency (RMD). Relative mycorrhizal dependency ranged widely among four zucchini hybrids. Gm inoculations had higher positive RMDs, while Gi inoculations had lower negative RMDs. Gm-inoculated Focus F₁ had the highest RMD (30.22 %). Arbuscular mycorrhizal fungi inoculated seedlings had wider cotyledons and stems. There were also significant effects of AMF inoculation on the most of nutrients.

Key words: AMF, colonization, nutrient uptake, relative mycorrhizal dependency symbiosis, zucchini.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are the most widespread root fungal symbionts and are associated with most plant species. Mycorrhizal symbiosis plays a significant role in the nutrition and development of host plants. Arbuscular mycorrhizal fungi (AMF) have been apparent to recover soil composition (Miller and Jastrow, 2000), and nutrient uptake of plants (Smith and Read 2008). Arbuscular mycorrhizal fungi (AMF) additionally support plants to cope with both biotic and abiotic stresses: they may fight with some soil-borne pathogens (Garmendia *et al.* 2004; Hu *et al.* 2010), minimize some nutrient deficiencies (especially phosphorous and micro nutrients), advance aridity, salinity, and pollution tolerance (Turkmen, *et al.* 2005; Sensoy, *et al.* 2007; Turkmen, *et al.* 2008).

Zucchini (*Cucurbita pepo* L.) is an important vegetable in Turkey and in the world (FAOSTAT 2009). So far, little research is done to reveal the effects of AMF on growth and yield of zucchini. Recent studies have suggested that AMF-inoculated zucchini could benefit from association with AMF (Colla *et al.*, 2008; Cardarelli *et al.*, 2010) by enhancing its nutrition in abiotic stress conditions.

Differences in mycorrhizal responsiveness among diverse crops and between ranges of genotypes within the same crop have also been established (Sensoy *et al.*, 2007; Meghvanski *et al.*, 2008; Miyauchi *et al.* 2008; Wang *et al.* 2008; Long *et al.* 2010). Sensoy *et al.* (2007) detected that there was a large variation in mycorrhizal colonization dependency among the pepper genotypes, and confirmed that appropriate cultivar-AMF

combinations have to be found in order to obtain the greatest gain from symbiosis.

Effectiveness of AMF is much better in a sterile growth medium (Wang *et al.*, 2008). Inoculation with AMF, in some vegetables, may improve growth performance (Temperini *et al.*, 2009). The benefits of AMF inoculation depend on the genotypic host-fungus combinations and also the type of the inoculums used (Rouphael *et al.* 2010). There has been insufficient attention to mycorrhizal response in the modern plant improvement activities, particularly in the development of hybrid varieties. Khalil *et al.* (1994) stated that modern cultivars in soybean showed lower growth response to mycorrhizal colonization than older cultivars.

Even under normal seedlings conditions, the effectiveness of different AMF on different zucchini hybrids has not been well documented. Therefore, the current study initiated to evaluate colonization, nutrient uptake, responsiveness and some seedling traits of four zucchini hybrids inoculated by three different AMF.

MATERIALS AND METHODS

Four zucchini hybrids Viz. Ezra F₁ (Multitarim Seed Company), 2: Natali F₁ (Anadolu Seed Company), 3: Focus F₁ (Beta Seed Company), 4: Comet F₁ (Beta Seed Company) used in protected cultivation in Turkey were examined. Three AMF inocula e.g. *Glomus intraradices* (Gi), *Glomus etinucatum* (Ge), and *Gigaspora margarita* (Gm) were used in the study. Inocula consisted of spores, extraradical mycelium and mycorrhizal roots.

Growth medium comprised of an autoclaved mixture of perlite and peat moss (1:1 v/v) in seedling

trays (Each individual plant cell measures approximately 4.5 cm L x 5.5 cm W x 5.5 cm D) covered by vermiculite. The experiment was conducted using an 4x4 factorial design (four zucchini hybrids, three AMF plus one control) with three random replications of fifteen cells each, for a total of 720 cells. One seed was sown per cell, each of which contained 80 cm³ of sterilized growth medium. In the AMF-inoculated samples, five g (25 spores g⁻¹) of inoculums were placed in the growth medium before the seeds were sown (Demir and Onogur, 1999). Seedling trays were placed in a growth chamber at a temperature of 22±2°C with 12 h fluorescent illumination (8,000 lux light intensity), and irrigated with distilled water. Each seedling was fertilized twice with a 5 ml of nutrient solution (for 1 L: 720 mg MgSO₄ 7.H₂O, 12.2 mg KH₂PO₄, 295 mg Ca(NO₃)₂.4H₂O, 240 mg KNO₃, 0.75 mg MnCl₂.4H₂O, 0.75 mg KI, 0.75 mg ZnSO₂.H₂O, 1.5 mg H₃BO₃, 0.001 mg CuSO₄.5H₂O, 4.3 mg FeNaEDTA, and 0.00017 mg Na₂MoO₄.2H₂O) modified by Vosatka and Gryndler (1999). Plants were harvested 9 weeks after seed sowing and inoculation.

Seedling emergence percentage, seedling emergence time, and true leaf emergence time were determined. Hypocotyledon height, cotyledon height, cotyledon length, and stem diameter were measured. Shoot heights, root lengths, stem diameters, leaf numbers, shoot dry weights, and root dry weights of seedlings were determined after harvesting. Samples were then oven-dried at 68 °C for 48 h, ground, and nitrogen (N) contents of shoots measured using Kjeldahl method; potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) contents of shoots measured using atomic absorption spectrophotometer ; phosphorous (P) contents of shoots measured using the vanadate-molybdate-yellow procedure with spectrophotometer (Kacar and Inal, 2008).

Zucchini roots were dyed to detect AMF presence using a modification of Phillips and Haymans (1970) method, and the percentage and intensity of mycorrhizal colonization was estimated using the gridline intersect method (Giovanetti and Mosse 1980). Relative mycorrhizal dependency (RMD) of zucchini hybrids was expressed as the difference between the total dry weight of the mycorrhizal plant and the total dry weight of the non-mycorrhizal plant as a percentage of the total dry weight of the mycorrhizal plant (Sensoy *et al.* 2007).

Data were analyzed using the SAS statistical program, with variance analysis conducted for all data. Differences between treatments were determined using Duncan's Multiple Range Test (SAS Software 1997).

RESULTS

Arbuscular mycorrhizal fungi application and arbuscular mycorrhizal fungi-zucchini hybrid interaction had significant effects on seedling emergence percentage

(Table 1). The lowest seedling emergence was observed in *Gi*-inoculated Ezra F₁ followed by -AMF Ezra F₁. On the other hand, there were no significant effects of AMF inoculation on seedling emergence time (Table 1). Hypocotyledon height and cotyledon height were significantly affected by genotype and AMF (Table 1). Arbuscular mycorrhizal fungi inoculated seedlings had shorter hypocotyledons, but wider cotyledons than those of non-inoculated seedlings. *Gi*-, *Ge*-, and *Gm*-inoculated seedlings had 6.1 %, 8.3 %, and 10.1 % shorter hypocotyledons than those of non-inoculated seedlings, respectively. On the other hand, *Gi*-, *Ge*-, and *Gm*-inoculated seedlings had 8.9 %, 16.1%, and 16.5 % wider cotyledons than those of non-inoculated seedlings, respectively. Although the seedlings of *Gi*-inoculated Ezra F₁, *Ge*-inoculated Natali F₁, and -AMF Natali F₁ had the shortest cotyledons, the seedlings of *Ge*-inoculated Comet F₁ had the longest cotyledons (Table1).

Arbuscular mycorrhizal fungi applications had significant effects on seedling stem diameter, but there were insignificant effects on leaf number, shoot height, and root length (Table 2). *Ge*- and *Gm*-inoculated zucchini seedlings had the maximum stem diameter, followed by *Gi*-inoculated ones. *Ge*-, *Gm*-, and *Gi*-inoculated seedlings had 7.2 %, 7.2%, and 4.3% wider stem diameters than those of non-inoculated seedlings, respectively.

Arbuscular mycorrhizal fungi applications had no significant effects on fresh and dry weights of zucchini seedling shoots and roots (Table 3). *Gi*-inoculated seedlings had the lowest shoot fresh and dry weight among the applications. *Gm*-inoculated zucchini seedlings had the highest root fresh and dry weight followed by *Ge*-inoculated ones, and then *Gi*-inoculated ones.

Although AMF applications had no significant effects on nitrogen (N) and magnesium (Mg) contents, there was significant genotypic effect on phosphorous (P), potassium (K) and calcium (Ca) contents (Table 4). While *Gi*-inoculated zucchini seedlings had the highest P content in shoots, *Gm*-inoculated ones had the lowest P content in shoots. Similarly, *Gi*-inoculated zucchini seedlings had the highest K content in shoots compared to the other applications. However, *Gm*-inoculated zucchini seedlings had the highest Ca content in shoots compared to the other applications. There were also significant zucchini hybrid × AMF interactions on P, K, Ca, and Mg contents

Although AMF applications had no significant effect on copper (Cu) and manganese (Mn) contents, there was significant mycorrhizal effect on iron (Fe) and Zinc (Zn) contents and mycorrhizal colonization (Table 5). While *Gi*-inoculated zucchini seedlings had the highest Fe and Zn contents, *Ge*-inoculated ones had the lowest Fe and Zn contents. Mycorrhizal colonization ranged from 24.40 % to 46.70 % among the treatments.

There were also significant zucchini hybrid \times AMF interactions on Fe, Zn, and Mn contents.

Relative mycorrhizal dependency (RMD) ranged widely among the zucchini hybrids tested (Figure 1). *Gm*-inoculations had positive RMDs on all hybrids. *Gm*-inoculated Focus F₁ had the highest RMD (30.22 %). *Ge*-inoculations had also positive RMDs except the Natali F₁. On the other hand, *Gi*-inoculations had all negative RMDs on all hybrids.

DISCUSSION

Many crops benefit from AMF in a variety of biotic and abiotic stress conditions, but several studies have shown that genetic variation in responses to AMF is obvious regardless of the crop (Sensoy *et al.*, 2007; Meghvanski *et al.*, 2008; Miyauchi *et al.*, 2008; Wang *et al.*, 2008; Long *et al.*, 2010). The limited number of studies on the effects of AMF in zucchini has suggested that AMF-inoculated zucchini could benefit from association with AMF in abiotic stress conditions (Colla *et al.*, 2008; Cardarelli *et al.*, 2010). Colla *et al.*, (2008) studied the effects of AMF on salt stressed zucchini plants grown at low and high phosphorous concentration. These researchers stated that the beneficial effects of AM on zucchini plants could be due to an improvement in water and nutritional status (high K and low Na accumulation). Cadradelli *et al.* (2010) studied the effects of AMF on alkaline stressed zucchini plants grown under mineral and organic fertilization. These researchers demonstrated that +AM plants under alkaline conditions had higher total, marketable yield and total biomass compared to -AM plants, which might be due to a better nutritional status. However, there has been no study on zucchini hybrid-AMF interaction. Wang *et al.* (2008) reported that the growth of cucumber seedlings was also influenced differently by the studied three AMF species. Their results indicated that growth of seedlings was significantly enhanced by *G. mossae*, inhibited by *G. veriforme*, and not significantly influenced by *G. intraradices*.

In the present study, the significant effects of AMF inoculation on the most of nutrients (especially on P, K, Ca, Fe, and Zn), cotyledon width and stem diameter were detected. Cardarelli *et al.* (2010) also demonstrated that +AM zucchini plants had higher P, K, Fe, Mn, and Zn and lower Na accumulation; +AM plants had also higher cotyledon, stem diameter, and root fresh weight compared to -AM plants. Wang *et al.* (2008) also found that N and P contents in cucumber roots and Mg, Cu, Zn contents in cucumber shoots were increased by inoculating AMF.

Relative mycorrhizal dependency (RMD) ranged widely (30.22% to -30.19%) among four zucchini hybrids with no clear correlation between mycorrhizal response and AMF colonization level and intensity. *Gm* inoculations had higher positive RMDs, while *Gi* inoculations had lower negative RMDs. *Gm*-inoculated Focus F₁ had the highest RMD (30.22 %). The finding of a lack of correlation between AMF colonization and RMD was similar to the findings of a study by Sensoy *et al.* (2007) that compared different pepper genotypes and AMF. Azcon *et al.* (1991) stated that there were differences in functional compatibility among AMF, even the root colonization was similar. There are variations in carbon demands of the different AMF species (Saikkonen *et al.* 1999). Wang *et al.* (2008) speculated that growth response induced by a specific AMF relied on recognition between AMF species and host plant; sometimes negative growth response by AMF induced by the imbalance of carbohydrate distribution between the shoots and roots of the plants. Hodge *et al.* (2010) stated that the major fluxes in AMF symbiosis appear to be of carbon from plant to fungus and phosphorous, and possibly nitrogen, from fungus to plant.

Temperini *et al.* (2009) stated that AMF inoculation showed improvement in transplant performance in local pepper genotypes. There are many other benefits of AMF in agriculture. AMF may positively affect crop development, even in phosphorous-rich soils, and the development of cultivars with improved symbiotic qualities would insure the production of good crop yields while improving agrosystem sustainability (Hamel and Strullu 2006).

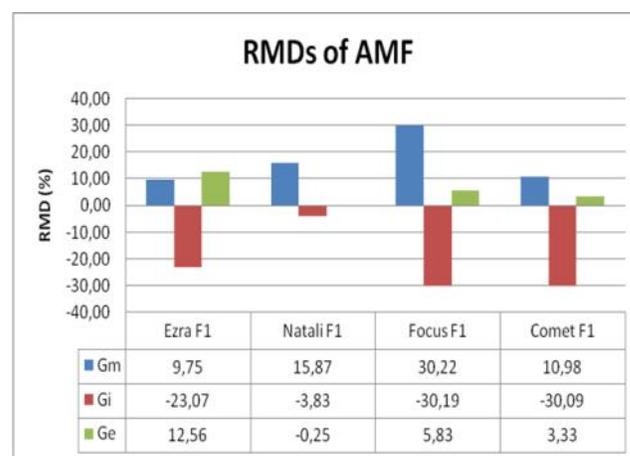


Figure 1. Relative mycorrhizal dependency (RMD) of four zucchini hybrids (Ezra, Natali, Focus, and Comet) inoculated with three AMF [*Glomus intraradices* (*Gi*), *Glomus etinucatum* (*Ge*), and *Gigaspora margarita* (*Gm*)].

Table 1. Effects of AMF [*Glomus intraradices* (*Gi*), *Glomus etinucatum* (*Ge*), and *Gigaspora margarita* (*Gm*)] and genotype on seedling emergence percentage, seedling emergence time, hypocotyledon height, cotyledon height, and cotyledon length of zucchini plants.

Applications		Seedling emergence (%)	Seedling emergence time (day)	Hypocotyledon height (mm)	Cotyledon width (mm)	Cotyledon length (mm)
Hybrids	Ezra F ₁	96.50 b**	7.75 a***	69.64 ab*	33.03 a***	50.52 b***
	Natali F ₁	100.00 a	5.89 b	74.98 a	29.37 b	49.26 b
	Focus F ₁	100.00 a	6.25 b	66.44 b	30.89 b	51.55 b
	Comet F ₁	100.00 a	6.56 b	70.54 ab	34.94 a	61.75 a
AMF	-AMF	98.83 ab*	6.06 NS	74.95 a*	29.02 c***	51.32 NS
	<i>Gm</i>	100.00 a	6.59	67.37 b	33.80 a	54.49
	<i>Gi</i>	96.67 b	6.96	70.38 ab	31.62 b	53.89
	<i>Ge</i>	100.00 a	6.72	68.75 b	33.68 a	52.61
Non inoculated	Ezra F ₁	95.33 ab*	7.03 NS	78.10 NS	30.57 NS	49.90 def**
<i>Gm</i> - inoculated	Ezra F ₁	100.00 a	8.14	64.67	35.13	52.37 c-f
<i>Gi</i> - inoculated	Ezra F ₁	90.67 b	8.22	74.00	30.03	47.27 f
<i>Ge</i> - inoculated	Ezra F ₁	100.00 a	7.38	61.80	36.37	52.53 c-f
Non inoculated	Natali F ₁	100.00 a	5.63	80.77	25.80	46.03 f
<i>Gm</i> - inoculated	Natali F ₁	100.00 a	5.92	69.80	29.33	48.83 def
<i>Gi</i> - inoculated	Natali F ₁	100.00 a	6.07	73.63	32.43	57.87 a-e
<i>Ge</i> - inoculated	Natali F ₁	100.00 a	5.97	75.70	29.90	44.30 f
Non inoculated	Focus F ₁	100.00 a	5.89	69.60	28.20	47.80 ef
<i>Gm</i> - inoculated	Focus F ₁	100.00 a	5.64	61.90	33.73	54.07 b-f
<i>Gi</i> - inoculated	Focus F ₁	100.00 a	6.67	67.63	28.83	51.63 c-f
<i>Ge</i> - inoculated	Focus F ₁	100.00 a	6.81	66.63	32.80	52.70 b-f
Non inoculated	Comet F ₁	100.00 a	6.02	71.33	31.50	61.53 abc
<i>Gm</i> - inoculated	Comet F ₁	100.00 a	6.65	73.10	37.00	62.70 ab
<i>Gi</i> - inoculated	Comet F ₁	100.00 a	6.91	66.23	35.17	58.80 a-d
<i>Ge</i> - inoculated	Comet F ₁	100.00 a	6.72	71.95	36.65	65.05 a

Values are the means of three replicate samples. AMF: Arbuscular Mycorrhizal Fungi. -AMF: Nonmycorrhizal plants. NS: nonsignificant. *:P<0.05; **:P<0.01; ***:P<0.001(significant)

Table 2. Effects of AMF [*Glomus intraradices* (*Gi*), *Glomus etinucatum* (*Ge*), and *Gigaspora margarita* (*Gm*)] and genotype on seedling stem diameter, leaf number, shoot height, and root length of zucchini plants.

Applications		Stem diameter (mm)	Leaf number	Shoot height (cm)	Root length (cm)
Hybrids	Ezra F ₁	3.45 c**	4.73 a***	13.02 NS	21.67 b*
	Natali F ₁	3.50 bc	4.89 a	13.25	21.04 b
	Focus F ₁	3.81 ab	4.77 a	12.43	24.21 a
	Comet F ₁	3.81 a	3.67 b	11.87	21.89 b
AMF	-AMF	3.44 b*	4.75 NS	13.31 NS	21.60 NS
	<i>Gm</i>	3.69 a	4.52	12.99	23.81
	<i>Gi</i>	3.59 ab	4.50	12.22	21.71
	<i>Ge</i>	3.69 a	4.37	12.05	21.68
Non inoculated	Ezra F ₁	3.09 NS	5.29 NS	13.76 NS	20.03 NS
<i>Gm</i> - inoculated	Ezra F ₁	3.43	4.55	12.08	22.37
<i>Gi</i> - inoculated	Ezra F ₁	3.61	4.46	13.19	21.04
<i>Ge</i> - inoculated	Ezra F ₁	3.68	4.61	13.03	23.23
Non inoculated	Natali F ₁	3.37	4.73	13.60	21.03
<i>Gm</i> - inoculated	Natali F ₁	3.72	4.99	14.60	21.96
<i>Gi</i> - inoculated	Natali F ₁	3.41	5.07	13.53	21.58
<i>Ge</i> - inoculated	Natali F ₁	3.49	4.77	11.25	19.59
Non inoculated	Focus F ₁	3.59	4.96	12.57	23.94
<i>Gm</i> - inoculated	Focus F ₁	3.70	4.70	12.73	26.13
<i>Gi</i> - inoculated	Focus F ₁	3.61	4.67	12.03	24.23
<i>Ge</i> - inoculated	Focus F ₁	3.75	4.76	12.39	22.55
Non inoculated	Comet F ₁	3.73	4.03	13.31	21.40
<i>Gm</i> - inoculated	Comet F ₁	3.94	3.83	12.55	24.76
<i>Gi</i> - inoculated	Comet F ₁	3.70	3.26	10.14	19.98
<i>Ge</i> - inoculated	Comet F ₁	3.89	3.49	11.25	21.19

Values are the means of three replicate samples. AMF: Arbuscular Mycorrhizal Fungi. -AMF: Nonmycorrhizal plants. NS: nonsignificant. *:P<0.05; **:P<0.01; ***:P<0.001(significant)

Table 3. Effects of AMF [*Glomus intraradices* (*Gi*), *Glomus etinucatum* (*Ge*), and *Gigaspora margarita* (*Gm*)] and zucchini hybrids on fresh and dry weights of shoots and roots.

Applications		Shoot fresh weight (g plant ⁻¹)	Root fresh weight (g plant ⁻¹)	Shoot dry weight (mg plant ⁻¹)	Root dry weight (mg plant ⁻¹)
Hybrids	Ezra F ₁	5.19 c**	0.53 NS	331 NS	21.57 NS
	Natali F ₁	5.47 bc	0.59	368	24.98
	Focus F ₁	6.02 ab	0.64	392	26.21
	Comet F ₁	6.58 a	0.65	383	26.19
AMF	-AMF	6.04 a**	0.49 c**	370 b**	19.36 b***
	<i>Gm</i>	6.34 a	0.71 a	442 a	30.67 a
	<i>Gi</i>	4.49 b	0.56 bc	283 c	20.86 b
	<i>Ge</i>	5.92 a	0.67 ab	379 b	28.07 a
Non inoculated Ezra F ₁		5.53 NS	0.39 NS	337 NS	15.30 NS
<i>Gm</i> - inoculated Ezra F ₁		5.35	0.55	367	23.38
<i>Gi</i> - inoculated Ezra F ₁		4.51	0.48	253	18.03
<i>Ge</i> - inoculated Ezra F ₁		5.38	0.70	367	29.56
Non inoculated Natali F ₁		5.51	0.46	360	19.21
<i>Gm</i> - inoculated Natali F ₁		6.08	0.79	417	33.72
<i>Gi</i> - inoculated Natali F ₁		4.73	0.49	343	21.70
<i>Ge</i> - inoculated Natali F ₁		5.59	0.63	353	25.28
Non inoculated Focus F ₁		6.07	0.56	377	22.29
<i>Gm</i> - inoculated Focus F ₁		6.74	0.75	537	35.19
<i>Gi</i> - inoculated Focus F ₁		5.17	0.63	257	21.76
<i>Ge</i> - inoculated Focus F ₁		6.11	0.62	397	25.58
Non inoculated Comet F ₁		7.03	0.55	407	20.61
<i>Gm</i> - inoculated Comet F ₁		7.18	0.73	450	30.37
<i>Gi</i> - inoculated Comet F ₁		5.31	0.62	277	21.94
<i>Ge</i> - inoculated Comet F ₁		6.93	0.76	410	31.83

Values are the means of three replicate samples. AMF: Arbuscular Mycorrhizal Fungi. -AMF: Nonmycorrhizal plants. NS: nonsignificant. **: $P < 0.01$; ***: $P < 0.001$ (significant)

Table 4. Effects of AMF [*Glomus intraradices* (*Gi*), *Glomus etinucatum* (*Ge*), and *Gigaspora margarita* (*Gm*)] and zucchini hybrids on nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) contents of shoots.

Applications		Nitrogen (%)	Phosphorous (%)	Potassium (%)	Calcium (%)	Magnesium (%)
Hybrids	Ezra F ₁	3.17 a ***	2.19 ***	4.13 a**	3.87 a***	1.20 NS
	Natali F ₁	2.87 a	0.85	3.31 b	2.55 b	1.47
	Focus F ₁	1.48 b	1.07	3.60 b	1.77 b	1.62
	Comet F ₁	1.40 b	0.57	3.28 b	1.67 b	1.68
AMF	-AMF	2.25 NS	1.07 bc ***	3.53 b***	1.97 b***	1.52 NS
	<i>Gm</i>	2.43	0.89 c	3.28 b	3.64 a	1.46
	<i>Gi</i>	2.11	1.35 a	4.27 a	2.38 b	1.54
	<i>Ge</i>	2.14	1.27 ab	3.25 b	1.82 b	1.51
Non inoculated Ezra F ₁		2.66 NS	1.77 c***	4.54 ab*	2.75 bc***	1.14 b**
<i>Gm</i> - inoculated Ezra F ₁		3.96	1.75 c	4.31 a-d	8.29 a	1.13 b
<i>Gi</i> - inoculated Ezra F ₁		2.96	2.15 b	4.49 a-d	3.08 bc	1.22 ab
<i>Ge</i> - inoculated Ezra F ₁		3.17	3.30 a	3.20 bcd	1.36 bc	1.41 ab
Non inoculated Natali F ₁		3.29	0.47 jk	3.00 bcd	0.60 c	1.20 ab
<i>Gm</i> - inoculated Natali F ₁		3.33	0.53 j	2.88 d	2.33 bc	1.43 ab
<i>Gi</i> - inoculated Natali F ₁		2.52	1.77 c	4.43 a-d	3.35 bc	1.07 b
<i>Ge</i> - inoculated Natali F ₁		2.21	0.67 hi	2.92 d	4.04 b	2.09 ab
Non inoculated Focus F ₁		1.90	1.63 d	3.16 bcd	1.87 bc	2.13 ab
<i>Gm</i> - inoculated Focus F ₁		1.22	0.73 h	2.99 bcd	2.13 bc	1.99 ab
<i>Gi</i> - inoculated Focus F ₁		1.30	1.27 e	4.85 a	2.74 bc	1.22 ab
<i>Ge</i> - inoculated Focus F ₁		1.40	0.63 i	3.36 a-d	0.66 c	1.13 b
Non inoculated Comet F ₁		1.29	0.43 k	3.39 a-d	2.33 bc	1.49 ab
<i>Gm</i> - inoculated Comet F ₁		1.71	0.80 g	2.93 cd	1.81 bc	1.28 ab
<i>Gi</i> - inoculated Comet F ₁		1.25	0.47 jk	3.39 a-d	0.77 c	2.54 a
<i>Ge</i> - inoculated Comet F ₁		1.22	0.90 f	3.62 a-d	1.98 bc	1.28 ab

Values are the means of three replicate samples. AMF: Arbuscular Mycorrhizal Fungi. -AMF: Nonmycorrhizal plants. NS: nonsignificant. **: $P < 0.01$; ***: $P < 0.001$ (significant)

Table 5. Effects of AMF [*Glomus intraradices* (*Gi*), *Glomus etinucatum* (*Ge*), and *Gigaspora margarita* (*Gm*)] and zucchini hybrids on mycorrhizal colonization and iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) contents contents of shoots.

Applications		Iron (mg kg ⁻¹)	Copper (mg kg ⁻¹)	Zinc (mg kg ⁻¹)	Manganese (mg kg ⁻¹)	Mycorrhizal colonization (%)
Hybrids	Ezra F ₁	148.56 a***	26.72 NS	70.868 a***	188.76 NS	24.80 NS
	Natali F ₁	108.84 b	26.39	48.99 b	200.24	30.51
	Focus F ₁	89.33 bc	22.61	65.79 a	197.17	27.21
	Comet F ₁	69.89 c	20.91	51.79 b	214.94	29.91
AMF	-AMF	86.59 bc***	25.05 NS	65.01 ab***	203.67 NS	0.00 b***
	<i>Gm</i>	94.46 b	22.46	57.76 b	195.29	31.61 a
	<i>Gi</i>	175.72 a	28.42	72.01 a	201.25	41.06 a
	<i>Ge</i>	56.10 c	19.39	42.88 c	199.29	40.66 a
Non inoculated Ezra F ₁		160.95 abc***	27.06 NS	89.14 a***	204.40 abc**	0.00 NS
<i>Gm</i> - inoculated Ezra F ₁		153.35 abc	24.85	82.12 ab	155.47 c	24.40
<i>Gi</i> - inoculated Ezra F ₁		213.01 ab	33.29	89.03 a	194.49 bc	37.10
<i>Ge</i> - inoculated Ezra F ₁		67.00 cde	21.66	23.17 d	200.67 bc	37.70
Non inoculated Natali F ₁		88.50 de	22.50	39.17 d	194.50 bc	0.00
<i>Gm</i> - inoculated Natali F ₁		57.00 de	24.00	46.00 cd	193.33 bc	35.11
<i>Gi</i> - inoculated Natali F ₁		189.29 ab	30.48	74.27 abc	195.47 bc	46.27
<i>Ge</i> - inoculated Natali F ₁		76.50 cde	28.50	35.50 d	217.67 ab	40.67
Non inoculated Focus F ₁		21.50 e	22.67	28.25 d	188.00 bc	0.00
<i>Gm</i> - inoculated Focus F ₁		39.17 de	20.17	51.17 bcd	184.00 bc	30.07
<i>Gi</i> - inoculated Focus F ₁		225.92 a	31.89	94.90 a	228.19 ab	34.17
<i>Ge</i> - inoculated Focus F ₁		27.50 e	12.25	76.33 abc	188.50 bc	44.60
Non inoculated Comet F ₁		38.25 de	27.99	93.02 a	227.79 ab	0.00
<i>Gm</i> - inoculated Comet F ₁		126.50 bcd	21.33	47.83 cd	248.33 a	36.87
<i>Gi</i> - inoculated Comet F ₁		74.67 cde	18.00	29.83 d	186.83 bc	46.70
<i>Ge</i> - inoculated Comet F ₁		37.75 de	14.00	36.50 d	187.75 bc	39.15

Values are the means of three replicate samples. AMF: Arbuscular Mycorrhizal Fungi. -AMF: Nonmycorrhizal plants. NS: nonsignificant. **: $P<0.01$, ***: $P<0.001$ (significant).

In conclusion, AMF offer valuable opportunities for the existing agricultural practice with regard to various biotic and abiotic stress conditions. Effective use of these symbiotic soil fungi is an essential element for sustainable agriculture. As shown in the examples, AMF may enhance seedling traits in vegetable species. Among the examined AMF, *Gigaspora margarita* followed by *Glomus etinucatum* (*Gm*) gave better RMDs for zucchini hybrids. Considering the wide variety of responses from different plant cultivars to AMFs, as demonstrated in this and other studies, appropriate cultivar-AMF combinations need to be identified in order to derive the utmost benefit from symbiosis. In the present study, *Gm*-inoculated Focus F₁ had the highest RMD (30.22 %). So far, modern plant breeding methods have paid little attention to such symbiotic relationships. Greater interest in this area, incorporating AMF-related traits from high-RMD genotypes into modern cultivars, could lead to improvements in zucchini production by increasing strength under various biotic and abiotic stress conditions that might be encountered during the crop production season. The determining the best AMF-cultivar combinations with improved symbiotic qualities would insure the production of good crop yields while improving agro-system sustainability. Growth response at seedling nursing could be a good indicator of AMF applications, but effectiveness of AMF on full growing

period of hybrids should also be investigated in depth for protected cultivation conditions in the future.

REFERENCES

- Azcon, R., R. Rubio, and J. M. Barea (1991). Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N₂-fixation (¹⁵N) and nutrition of *Medicago sativa* L. New Phytol., 117:399–404.
- Cardarelli, M., Y. Roupahel, E. Rea, and G. Colla (2010). Mitigation of alkaline stress by arbuscular mycorrhiza in zucchini plants grown under mineral and organic fertilization. J. Plant Nutrition & Soil Sci., 173: 778–787.
- Colla, G., Y. Roupahel, M. Cardarelli, M. Tullio, C.M. Rivera, and E. Rea (2008). Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. Biol. & Fertility of Soils, 44(3):501-509.
- Demir, S. and E. Onogur (1999). *Glomus intraradices* Schenck&Smith: A hopeful vesicular-arbuscular mycorrhizal (VAM) fungus determined in soils of Türkiye. J. Turk. Phytopatol., 28:33-34.
- FAOSTAT, (2009) Statistic Database. <http://apps.fao.org/>

- Garmendia, I., N. Goicoechea, and J. Aguirolea (2004). Effectiveness of three *Glomus* species in protecting pepper (*Capsicum annuum* L.) against verticillium wilt. *Biological Control*, 31:296-305.
- Giovanetti, M. and B. Mosse (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.*, 84: 489-500.
- Hamel, C. and D. G. Strullu (2006). Arbuscular mycorrhizal fungi in field crop production: Potential and new direction. *Canadian J. Plant Sci.*, 86(4):941-950.
- Hodge, A., T. Helgason, and A. H. Fitter (2010). Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology*, 3(4):267-273.
- Hu, J., X. Lin, J. Wang, W. Shen, S. Wu, S. Peng, and T. Mao (2010). Arbuscular mycorrhizal fungal inoculation enhances suppression of cucumber fusarium wilt in greenhouse soils. *Pedosphere*, 20(5), 586-593.
- Kacar, B. and A. Inal (2008). *Plant Analysis*. Nobel Publication, Ankara, ISBN: 978-605-395-036-3.
- Khalil, S., T. E. Loynachan, and M. A. Tabatabai (1994). Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. *Agron. J.*, 86(6), 949-958.
- Long, L. K., Q. Yao, Y. H. Huang, R. H. Yang, J. Guo, and H. H. Zhu (2010). Effects of arbuscular mycorrhizal fungi on zinnia and the different colonization between *Gigaspora* and *Glomus*. *World J. Microbiol.*, 26:1527-1531.
- Meghvanski, M. K., K. Prasad, D. Harwani, and S.K. Mahna (2008). Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. *Eur. J. Soil Biol.*, 44: 316-323.
- Miller, R. M. and J. D. Jastrow (2000). Mycorrhizal fungi influence soil structure. In: Kapulnik Y, Douds Jr. DD (Eds.), *Arbuscular Mycorrhizas: Physiology and Function*. Kluwer Academic Publication, pp. 3-18.
- Miyauchi, M. Y.H., D. S. Lima, M. A. Nogueira, G. M. Lovato, L. S. Murate, M. F. Cruz, J. M. Ferreira, and G. Andrade (2008). Interactions between diazotrophic bacteria and mycorrhizal fungus in maize genotypes *Scientia Agricola*, 65(5): 525-531.
- Phillips, J. M. and D. S. Hayman (1970). Improved procedure for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 55: 158-161.
- Rouphael, Y., M. Cardarelli, E. Di Mattia, M. Tullio, E. Rea, and G. Colla, (2010). Enhancement of alkalinity tolerance in two cucumber genotypes inoculated with an arbuscular mycorrhizal biofertilizer containing *Glomus intraradices*. *Biol. Fertil. Soil*, 46(5):499-509.
- Saikkonen, K., U. Ahonen-Jonnarth, A. M. Markkola, M. Helander, J. Tuomi, M. Roitto, and H. Ranta (1999). Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and below-ground sinks. *Ecology Letters*, 2: 19-26.
- Sensoy, S., S. Demir, O. Turkmen, C. Erdinc, and O. B. Savur (2007). Responses of some different pepper (*Capsicum annuum* L.) genotypes to inoculation with two different arbuscular mycorrhizal fungi. *Sci. Hort.*, 113(1):92-95.
- SAS Software, (1997) SAS Institute Inc., Cary, NC, USA.
- Smith, S. E. and D. M. Read (2008). *Mycorrhizal Symbiosis*, 3rd Edition. Academic Press, London.
- Temperini, O., Y. Rouphael, L. Parrano, E. Biagiola, G. Colla, R. Mariotti, E. Rea, and C.M. Rivera (2009). Nursery inoculation of pepper with arbuscular mycorrhizal fungi: An effective tool to enhance transplant performance. *Acta Hort.* (ISHS), 807:591-596.
- Turkmen, O., S. Demir, S. Sensoy, and A. Dursun (2005). Effects of arbuscular mycorrhizal fungus and humic acid on the seedling development and nutrient content of pepper grown under saline soil conditions. *J. Biol. Sci.*, 5(5):568-574.
- Turkmen, O., S. Sensoy, S. Demir, and C. Erdinc (2008). Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress. *Afr. J. Biotech.*, 7(4):392-396.
- Vosatka, V. and M. Gryndler (1999) Treatment with culture fractions from *Pseudomonas putida* modifies the development of *Glomus fistulosum* mycorrhiza and the response of potato and maize plants to inoculation. *Appl. Soil Ecol.*, 11: 245-251.
- Wang, C., X. Li, J. Zhou, G. Wang, and Y. Dong (2008). Effects of arbuscular mycorrhizal fungi on growth and yield of cucumber plants. *Commun. Soil Sci. Plant Anal.*, 39(3-4):499-509.