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Effects on growth of persimmon (*Diospyros virginiana*) rootstock of arbuscular mycorrhizal fungi species

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Abstract: Persimmon (*Diospyros kaki* Thunb.) is grown in many parts of the world that display subtropical climate conditions, including Turkey. There are 2 common rootstocks used in its production: *D. kaki* and *D. virginiana* Thunb. Arbuscular mycorrhiza (AM), a symbiosis between plant roots and members of an ancient phylum of fungi, Glomeromycota, improves root development, water supply, and nutrients such as phosphate and zinc in the host plant. In this study, the effects of 5 AM fungi species (*Glomus mosseae*, *G. clarium*, *G. etunicatum*, *G. caledonium*, and *G. intraradices*) on plant growth, chlorophyll concentration, and chlorophyll fluorescence (*Fv'/Fm'*) in *D. virginiana* were investigated under greenhouse conditions. We determined that mycorrhizal inoculations increased shoot and root dry weight compared to the noninoculated plants. Plants inoculated with *G. etunicatum* showed the highest total plant dry weight. Highest leaf chlorophyll concentration was measured in a plant inoculated with *G. caledonium*. The results of chlorophyll fluorescence were similar for all AM inoculations; however, they significantly differed from those of noninoculated plants. The results demonstrated the benefit potential of mycorrhizal inoculations for persimmon production.

Key words: Persimmon, plant development, chlorophyll fluorescence, mycorrhiza species, root colonization

1. Introduction

Persimmon originated from China and spread to Korea and Japan. Its cultivation in Western countries was initiated in the second half of the 19th century. In recent years, there has been renewed interest in the cultivation of persimmon in various countries of the Mediterranean basin, including Turkey. Turkey's persimmon production is 28,295 t in 2090 planted orchards (http://tuik.gov.tr/). Most Mediterranean persimmon production is based on the cultivars Kaki Tipo, Rojo Brillante, and Triumph. Other cultivars were introduced from Japan to local research institutions in Italy, Israel, Spain, and Turkey (Giordani, 2002). Although *D. kaki* is the most popular persimmon rootstock in Turkey, *D. virginiana*, which originates from North America, is increasing in popularity. It can be easily propagated by rootstock and is better adapted to poorly drained clay soils than *D. kaki*. *D. virginiana* is called simmon, possumwood, and Florida persimmon and is a slow-growing tree of moderate size found on a wide variety of soils and sites. *D. virginiana* rootstock is compatible with many cultivars, including Fuyu in California (Hodgson, 1939; Halls, 1990).

Arbuscular mycorrhizal (AM) fungi belong to the order Glomales and are most abundant in agricultural soil. They establish a mutualistic symbiosis with the roots of approximately 90% of terrestrial plant species, where plant photosynthates are exchanged for water and mineral resources acquired by the fungi from the soil (Selosse et al., 2006; Smith and Read, 2008; Zou et al., 2013). Mycorrhizal associations between a fungus and a plant root are ubiquitous in the natural environment (Hodge, 2000). Seven different categories of mycorrhizal symbiosis have been distinguished on the basis of their morphological characteristics and the fungal and plant species involved. AM is regarded as the most ancient and widespread form (Finlay, 2008). There are several species of mycorrhiza and one form, *Glomus mosseae*, is well known for colonizing several vegetables, fruits, cereals, and industrial crops (Ndiaye et al., 2011; Ortas et al., 2011; Naher et al., 2013).

The main functions of AM fungi on plants are 1) promoting the absorption of minerals, especially P, Zn, Cu, and NH_4; 2) increasing water uptake; 3) stimulating growth; 4) producing high-quality fruits; 5) enhancing resistance to environmental stresses; and 6) enhancing resistance to soil disease. Mycorrhizae produce many effects on plants of horticultural value (Ortas and Varma, 2007; Ortas et al., 2011; Zou et al., 2013). The fungi can

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increase seedling survival rate, plant growth rate, and the number of flowers produced (Ortas et al., 2011). Mycorrhizae also increase citrus seedling quality and improve growth after transplantation from greenhouse to field conditions (Ortas, 2012b). However, they have not been well implemented in persimmon cultivation. The present study evaluates the effects of 5 AM species on plant growth, chlorophyll concentrations, and chlorophyll fluorescence in *D. virginiana* at seedling stage.

2. Materials and methods

2.1. Experimental details

The experiments were carried out in greenhouse conditions at the Çukurova University Research Farm in Adana, Turkey. Mycorrhizal and nonmycorrhizal seedlings were produced under greenhouse conditions. The plants were grown in a greenhouse at 30–35 °C and a relative humidity of 70%–85% with a 16-h day and 8-h dark photoperiod between June and October 2012. Pots were surface-sterilized with ethanol prior to being filled with the growth media explained below. *Diospyros virginiana* was used as a test plant. *D. virginiana* seeds were surface-sterilized with sodium hypochlorite solution (1% available chlorine) for 10 min, rinsed 3 times, soaked in distilled water, and then planted in the pots. Eight weeks after seed sowing, uniform seedlings at the 3 and 4 true-leaf stages were divided into 6 treatment groups, 5 AM inoculated and 1 noninoculated, and transplanted into 3-L plastic pots. One seedling was transplanted to each pot. The pots were periodically and manually watered to keep the soil moisture at approximately 80% of field capacity.

2.2. Mycorrhiza inoculation and growth medium

Approximately 1000 spores from each mycorrhizae inoculum species were applied per plant. Supplied from Rothamsted Research, UK, *G. mosseae* (Nicolson and Gerdemann), *G. caledonium* (Nicolson and Gerdemann), *G. etunicatum* (Becker and Gerdemann), *G. clarium* (Nicolson and Schenck), and *G. intraradices* (Schenck and Smith) mycorrhizae species were used and added to the medium 30 mm below the seedling roots. Control seedlings were transplanted into the growth medium without an inoculum. The experiment was performed in andesitic tuff, soil, and compost (6:3:1 v/v) mixture. Soil material was collected from surface horizons of clay loam Menzilat soil series (0–20 cm) (Typic Xerofluvents*)* in the Çukurova Basin (southern Turkey), displaying 7.45 pH, low organic matter content (1.41%), high $CaCO_3$ content (28%), and 0.5 M $NaHCO_3$ (pH 8.5) 45.5 kg ha^{-1} extractable P. Growth medium was autoclaved at 121 °C for 2 h prior to use. Three-liter pots were used under greenhouse conditions.

2.3. Leaf chlorophyll concentration and fluorescence measurements

Chlorophyll concentration and maximum chlorophyll fluorescence efficiency in light-adapted stage were measured on 2 fully expanded young leaves (third and fourth leaves from the shoot apex) of each replicate. Chlorophyll levels were estimated using a SPAD portable apparatus (Minolta Co., Japan) and chlorophyll fluorescence parameters (*Fv'/Fm'*) were measured with a portable fluorimeter (Photon System Instruments Ltd., Czech Republic).

2.4. Growth parameters and root colonization

The plants were harvested and the leaf number and stem length per plant were measured. In order to determine dry mass production, the plants were separated into shoots (all leaves and stem) and roots. Plants were dried at 72 °C until their weight was stabilized using a thermoventilated oven. The dry weight of shoots and roots was recorded at harvest. Collected roots were separated from the growth medium by washing with distilled water. Roots were dried on a tissue paper. Prior to drying, subsamples of about 0.5 g were extracted from the roots and preserved in a mixture of ethanol, glacial acetic acid, and formalin for the determination of root length and mycorrhizal infection. Roots were stained as described by Koske and Gamma (1989) and mycorrhizal infection was determined using the method of Giovannetti and Mosse (1980).

2.5. Mycorrhizal dependency

Mycorrhizal dependency (MD) was determined by expressing the difference between the dry weight of the mycorrhizal plant and the dry weight of the nonmycorrhizal plant as a percentage of the dry weight of the mycorrhizal plant (Plenchette et al., 1983).

2.6. Statistical analysis

The experiment was arranged as a complete randomized design with 10 replicates for each control and AM treatment. AM root colonization percentage was transformed to arc sin for data analysis. Data were analyzed by ANOVA and the main effects of different mycorrhizal inoculations were separated by Fisher's LSD test at $P \leq 0.05$. The correlation coefficients between all measured characteristics were also calculated. All statistical analyses were performed using SAS v9.00 statistics software (SAS Institute, USA). SigmaPlot version 11.00 (SYSTAT Software, USA) was used for graphical data presentation.

3. Results

3.1. Root colonization

Plant root colonization level was significantly affected by mycorrhizal inoculation (28%–40%) (Table 1). *G. caledonium* and *G. intraradices* showed more intensive root colonization (40%) than other inocula. Among the other mycorrhizal species, *G. clarium* had the lowest percentage

Table 1. Root colonization, plant height, and leaf number of *D. virginiana* seedlings inoculated with different mycorrhizal species.

Mycorrhizal species	% Root colonization		Plant height (cm)	Leaf number
	%	transformed to arc sin		
G. clarium	28	31.76 ± 2.39 b	39.16 ± 2.81 a	33.00 ± 3.94 a
G. etunicatum	36	36.65 ± 3.14 ab	39.74 ± 4.59 a	31.25 ± 2.29 ab
G. mosseae	30	33.09 ± 2.00 ab	38.80 ± 5.02 a	32.50 ± 1.32 a
G. caledonium	40	39.18 ± 1.86 a	32.94 ± 2.29 ab	32.25 ± 2.01 a
G. intraradices	40	39.18 ± 1.86 a	25.40 ± 1.06 b	23.75 ± 1.68 c
Control	0	0.00 ± 0.00 c	28.24 ± 2.01 b	24.40 ± 2.29 bc
Prob > f	-	<0.0001	0.0153	0.0251
$LSD_{5\%}$	-	6.133	9.574	7.011

Mean values ± standard deviations with the same letters are not significantly different (LSD test, P < 0.05).

of root colonization on *D. virginiana* seedlings. Since the growth medium was sterilized, control treatment showed no infection.

3.2. Plant growth parameters

Mycorrhizal inoculation significantly increased plant height, leaf number, plant dry root, dry shoot weight, and total dry weight for most mycorrhizal species tested (Tables 1 and 2). *G. etunicatum*-, *G. clarium*-, and *G. mosseae*-inoculated plants produced the tallest plants, while *G. clarium*-, *G. mosseae*-, and *G. caledonium*-inoculated plants produced the greatest leaf numbers per plant. In contrast, *G. intraradices*-inoculated plants produced the lowest plant height and the least number of leaves (Table 1).

AM symbiosis also increased plant shoot and root dry weight compared to the control. In particular, colonization by *G. etunicatum* significantly promoted shoot and root dry weight in *D. virginiana* seedlings (Table 2). Shoot dry weight (SDW) ranged from 4.01 to 7.61 g pot^{-1}, root dry weight (RDW) ranged from 1.74 to 3.59 g pot^{-1}, and total dry weight (TDW) ranged between 5.75 and 11.20 g pot^{-1}. *G. etunicatum*-inoculated plants produced the highest TDW, whereas noninoculated plants produced the lowest. Shoot/root DW ratio was slightly affected by AM symbiosis. All AM species had the same effect on shoot/root DW except *G. intraradices*. This would suggest that *G. intraradices* did not promote plant growth on *D. virginiana*.

MD was calculated. *G. etunicatum*-inoculated plants showed 48.66% dependency, and *G. clarium*- and *G. caledonium*-inoculated plants showed 40.04% and 40.23% dependency, respectively (Table 2).

3.3. Leaf chlorophyll concentration and fluorescence

AM species significantly affected leaf chlorophyll (Chl) concentration (Figure 1a). Leaf chlorophyll concentration ranged between 32.65 and 36.60. The highest Chl

Table 2. Shoot, root, total, and shoot/root dry weight and mycorrhizal dependency (%) of *D. virginiana* seedlings inoculated with different mycorrhizal species.

Mycorrhizal species	SDW (g)	RDW (g)	SDW/RDW	TDW (g)	MD (%)
G. clarium	6.90 ± 1.19 ab	2.69 ± 0.38 ab	2.57 ± 0.21 a	9.59 ± 1.54 a	40.04
G. etunicatum	7.61 ± 0.95 a	3.59 ± 0.54 a	2.12 ± 0.22 a	11.20 ± 1.40 a	48.66
G. mosseae	6.93 ± 0.23 ab	2.75 ± 0.08 ab	2.52 ± 0.14 a	9.68 ± 0.20 a	40.60
G. caledonium	6.49 ± 0.89 ab	3.13 ± 0.37 a	2.07 ± 0.17 a	9.62 ± 1.23 a	40.23
G. intraradices	4.85 ± 0.40 bc	3.54 ± 0.29 a	1.37 ± 0.03 b	8.39 ± 0.69 ab	31.47
Control	4.01 ± 0.65 c	1.74 ± 0.25 b	2.31 ± 0.15 a	5.75 ± 0.90 b	-
Prob > f	0.0276	0.0105	0.0040	0.0378	-
$LSD_{5\%}$	2.314	1.017	0.484	3.190	-

Mean values ± standard deviations with the same letters are not significantly different (LSD test, P < 0.05).

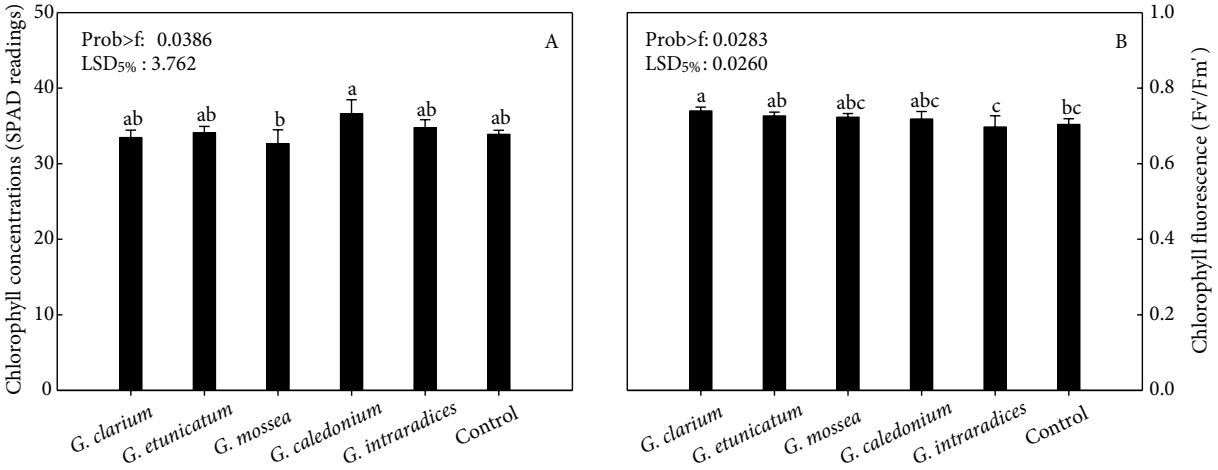

Figure 1. Chlorophyll concentration **(a)** and chlorophyll fluorescence **(b)** of *D. virginiana* seedlings. Bars indicate standard deviations. Those with the same letters are not significantly different.

concentration was obtained from the leaves of plants inoculated with *G. caledonium*, whereas the lowest was obtained from *G. mosseae*-inoculated plants.

Chlorophyll fluorescence was significantly affected by mycorrhizae species and changed from 0.6970 to 0.7397 (Figure 1b). The leaves of *G. clarium*-inoculated plants had the highest chlorophyll fluorescence of all inoculated and control plants. The lowest chlorophyll fluorescence was observed in *G. intraradices*-inoculated plants.

3.4. Correlation coefficients analysis

Significant correlations between the investigated variables were determined with the exception of SPAD and chlorophyll fluorescence (Table 3). Additionally, the correlation coefficients between % root colonization and TDW (0.49), RDW (0.58), and SDW (0.40) were statistically significant. High correlation coefficients were also obtained between plant height and leaf number (0.62), SDW (0.68), SDW/RDW (0.65), TDW (0.56), and

Table 3. Correlation coefficients analysis of investigated variables for *D. virginiana* seedlings inoculated with different mycorrhizal species.

Character	% RC	Plant height	Leaf number	SDW	RDW	SDW/RDW	TDW	SPAD	PSII
% RC	1.00	0.18^{ns}	0.30^{ns}	0.40^{*}	0.58^{***}	-0.24^{ns}	0.49^{**}	0.01^{ns}	0.15^{ns}
Plant height		1.00	0.62^{***}	0.68^{***}	0.18^{ns}	0.65^{***}	0.56^{**}	-0.29^{ns}	0.42^{*}
Leaf number			1.00	0.87^{***}	0.47^{**}	0.46^{*}	0.80^{***}	-0.11^{ns}	0.42^{*}
SDW				1.00	0.68^{***}	0.38^{*}	0.97^{***}	-0.18^{ns}	0.41^{*}
RDW					1.00	-0.39^{*}	0.84^{***}	0.13^{ns}	0.12^{ns}
SDW/RDW						1.00	0.15^{ns}	-0.38^{*}	0.41^{*}
TDW							1.00	-0.09^{ns}	0.34^{ns}
SPAD								1.00	-0.07^{ns}
PSII									1.00
Mean	29.98	34.05	29.53	6.13	2.91	2.18	9.04	34.24	0.72
St. dev.	14.57	8.80	6.29	2.07	0.95	0.52	2.80	2.91	0.02
N	30	30	30	30	30	30	30	30	30

***: P < 0.001, **: P < 0.01, *: P < 0.05, ns: not significant.

chlorophyll fluorescence (0.42). In contrast, a negative correlation was determined between SDW/RDW and SPAD (–0.38).

4. Discussion

In the present study we described how different AM species affected the seedling development of *D. virginiana* by evaluating plant growth characteristics, mycorrhizal colonization, MD, leaf chlorophyll concentration, and fluorescence. Significant differences were observed in plant growth of persimmon seedlings inoculated with different AM species. *G. caledonium*- and *G. intraradices*-inoculated seedlings showed up to 40% colonization. If the growth medium was partially sterilized, no root colonization was observed in the control seedlings. Ortas et al. (2011) and Ortas and Akpinar (2011) previously used the same inoculum for several plant species and they reported that mycorrhizal inoculation significantly influenced plant root colonization.

Our results suggest that mycorrhizae species have different influences on the plant growth characteristics tested in our experiment. *G. etunicatum*-inoculated seedling produced more SDW and RDW than other species. Matsubara and Hosokawa (1999) inoculated *D. kaki* seedlings from seeds with different species of AM fungi, such as *G. margarita*, *G. aggregatum*, *G. fasciculatum*, and *G. mosseae*, and they observed that mycorrhizae-inoculated plantlets increased their vegetative growth (SDW, RDW, leaf number, and leaf area) with most inocula. On the other hand, Ortas et al. (2011) claimed that mycorrhizal inoculation, in all the species assayed, resulted in a significant increase in plant shoot and dry weight. Similar results were reported in *D. kaki* (Matsubara and Hosokawa, 1999), pepper (Waterer and Coltman, 1989; Ortas et al., 2011), cassava (Oyetunji et al., 2007), peach (Wu et al., 2011), and citrus (Ortas et al., 2002). Marin et al. (2003) studied the effect of several AM inoculum seedlings on persimmon Rojo Brillante and found that *G. intraradices* significantly increased shoot height compared to the control and *G. mosseae* treatments. In this study, the mycorrhizae-inoculated *D. virginiana* seedling produced more TDW than the control plants. Similarly, the *G. etunicatum*-inoculated seedling produced more SDW than that of *G. mosseae* inoculation.

MD was calculated and found to be highest for *G. etunicatum* (48.66), followed by *G. caledonium* (40.23) and *G. mosseae* (40.60). Saggin-Junior and de Silva (2006) and Ortas (2012a) postulated that horticultural plants are mycorrhizal-dependent. It is therefore necessary to evaluate the MD of seedlings in order to obtain an efficient selection of AM.

AM species significantly affected the leaf chlorophyll concentration of *D. virginiana*. Manoharan et al. (2008) reported that the total chlorophyll content increased in *Cassia siamea*, *Delonix regia*, *Erythrina variegata*, *Samanea saman*, and *Sterculia foetida* in mycorrhizal inoculated seedlings compared to noninoculated plants. Cho et al. (2009) indicated that the chlorophyll content of citrus seedlings was significantly enhanced by AM inoculations compared to the noninoculated seedlings. Bhattacharjee and Sharma (2012) also claimed that chlorophyll contents in the AM-treated plants were higher than the control in pigeon pea. Wu and Zou (2010) indicated that the beneficial effects of mycorrhiza could contribute to high chlorophyll and therefore high photosynthetic activity. In addition, Demir (2004) reported that *G. intraradices* increased the chlorophyll concentration of *Capsicum annuum*.

Chlorophyll fluorescence measurements are a nondestructive, noninvasive, and reliable tool to identify the first signals of plant–mycorrhizal fungi interaction (Corrêa et al., 2006). It is well known that the concentration of chlorophyll is associated with photosynthetic rate, and the characterization of chlorophyll fluorescence reflects the state of the photosynthetic apparatus (Zhu et al., 2012). Potential quantum yield of PSII (*Fv/Fm*) is higher in plants with a higher degree of mycorrhizal colonization, especially in suboptimal conditions (Pinior et al., 2005). On the other hand, chlorophyll fluorescence was proven to be a very useful noninvasive tool for the evaluation of the effect of environmental stresses (such as salinity, temperature, and water deficit) on photosynthetic properties (Oyetunji et al., 2007; Zhu et al., 2012).

Table 3 shows that there is a significant correlation between root colonization and plant growth characteristics. It seems that *G. etunicatum* is one of the leading inocula for *D. virginiana* seedling production. Further studies need to be conducted under field conditions.

In conclusion, the results of the present study indicated that inoculation with various mycorrhizal species has positive effects on the development of *D. virginiana* seedlings. *G. etunicatum* remarkably promoted seedling growth and development. Compared to noninoculated plants, mycorrhizal inoculation increased shoot and root dry weight. In addition, there was a slight increase in leaf chlorophyll concentration and fluorescence by *G. caledonium* and *G. clarium* inoculations, respectively. The results are promising and point to a practical usage of mycorrhizal inoculation for the production of healthy *D. virginiana* seedlings. Furthermore, the markedly positive effects of AM species on plant growth should be beneficial for plant nurseries producing persimmon seedlings. Finally, it would also be useful to establish mycorrhizal inoculation experiments under field conditions.

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