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Effects of vesicular - arbuscular mycorrhizal (VAM) fungi on the seedling growth of three *Pistacia* species

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Abstract: *The experiment was undertaken to test the efficiency of inoculation of vesicular-arbuscular mycorrhizal (VAM) fungi on the seedling growth of three Pistacia species used as rootstocks. The stratified Pistacia seeds were inoculated with VAM fungi. The highest rate of inoculated roots was 96.7 % in P. khinjuck seedlings with G. clarum and G. etunicatum, 83.3 % in P. vera seedlings with G. caledonium and 73.3 % in P. terebinthus seedlings with G. caledonium. Mycorrhizal inoculations improved seedling height only in P. terebinthus. Certain mycorrhizal inoculations increased the leaf N, but not P and K contents. Seedlings inoculated with G. caledonium had higher reducing sugar contents. It was concluded that pre-inoculated Pistacia seedlings could have a better growth in the harsh field conditions.*

Key words: *VAM fungi, Mycorrhiza, Pistacia, Growth, Nutritional status.*

Introduction

Mycorrhizas are widespread associations characterized by a bi-directional transfer of nutrients between plants and the associated fungi, where plants provide sugar to the fungi and the fungi help plants on the acquisition of mineral nutrients from the soil (Smith and Barker, 2002). Beneficial interactions between vesicular-arbuscular mycorrhizal (VAM) fungi and horticultural crops have been well documented (Menge, 1983). For a normal plant growth, fruit trees exhibit strong dependency on this symbiosis and can develop VAM relationship in natural growing conditions. But, during the initial stages of recent nursery tree production, plants are generally grown in fumigated or fungicide-treated soils to avoid destructive pathogens such as *Phytophthora sp*. and *Phyhium sp*. (Fontanet *et al.*, 1998). Concomitantly, the fumigated nursery beds, potting substrates or field plots usually lack VAM fungi. A survey conducted in the Italian citrus nurseries showed that the absence of VAM fungi in many samples could be due to common use of fumigant nematicedes (Inserra *et al.*, 1980).

The poor initial growth and/or low transplanting success of nursery plants were often related to the lack of VAM fungi in the sterilized growing media used in the nursery or in fumigated field plots (Timmer and Leyden, 1978; Menge *et al.*, 1980; Matosevic *et al.*, 1997; Linderman and Davis, 2001). The beneficial effects of incorporating VAM fungi into the sterile growing media used for nursery plant production were reported for various horticultural plants such as the peach (LaRue *et al.*, 1975), avocado (Menge *et al.*, 1980) citrus (Camprubí and Calvet, 1996), strawberry (de Silva *et al.*, 1996) and woody ornamentals (Hall and Bowes, 1984).

Pistachio nursery tree production is done by budding the desired pistachio cultivars onto various seedling rootstocks those belong to *Pistacia sp*. But, the growth of *Pistacia* seedlings in the nursery is considerably slow to reach the sufficient diameter for budding in the first year, and the transplantation success of the nursery trees in the field is much lower than those of other deciduous fruit trees. The fumigation of the growing media used in the container-grown pistachio nursery tree production might cause these problems. Because, *Pistacia* is known to be VAM-dependent (Schubert and Martinelli, 1988). However, little is known about the response of *Pistacia* species to VAM fungi inoculation (Schubert and Martinelli, 1988; Camprubi *et al.*, 1992). The inoculation of *Pistacia* seedlings with VAM fungi at the pistachio nurseries may be beneficial to obtain a better tree performance as seen in other horticultural plants. This study was conducted to investigate the effects of mycorrhizal inoculations on the seedling growth of three *Pistacia* species used as rootstocks in the pistachio nursery tree production.

Materials and Methods

The experiment was carried out in a greenhouse at the Pistachio Research Institute, Gaziantep, Turkey, using three *Pistacia* species (*P. vera, P. khinjuck* and *P. terebinthus*) and five mycorrhizal treatments (*Glomus etunicatum*, *Glomus caledonium, Glomus clarum, Glomus mosseae* and mixed inoculum). Mixed inoculum consisted of equal spore mixture of *Glomus etunicatum*, *Glomus caledonium, Glomus clarum, Glomus mosseae, Glomus fasciculatum* and *Dr. Kindom.* The experiment also included a non-mycorrhizal treatment for all *Pistacia* species tested.

Pistacia seedling production: Seeds were collected from the open pollinated trees of selected genotypes: *P. vera* seeds from cv Siirt V53, *P. khinjuck* seeds from buttum type 0B5 and *P. terebinthus* seeds from terebinthus type 63 ME 01. The seeds were calibrated, and the average seed weight was 1.2 g for *P. vera,* 0.2 g for *P. khinjuck* and 0.1 g for *P. terebinthus*. At the first week of December, the hulled seeds of *P. khinjuck* and *P. terebinthus* were treated with 250 ppm GA_3 for 24 hr in order to

Table – 1 : Percentage of inoculated roots in *Pistacia* seedlings.

Inoculation treatments	Percentage of inoculated roots		
	P. vera	*P. khinjuk*	*P. terebinthus*
G. caledonium	83.3	93.3	73.3 a [z]
G. clarum	73.3	96.7	53.3 ab
G. etunicatum	53.3	96.7	50.0 ab
G. mosseae	80.0	80.0	56.7 ab
Mixed	80.0	93.3	36.7 b

[z]: Mean separation in columns by Duncan MRT, $p \leq 0.05$.

ease the stratification. All seeds were stratified under outdoor condition for 40 days in a box filled with perlite. The stratified seeds were then put into heated greenhouse (20 ºC day/ 18 ºC night) for one week to induce root development.

Inoculation: The fumigated growing media prepared for nursery tree production of Pistachio Research Institute was used in the experiment. The mycorrhizal soils were provided by the Soil Science Department of Cukurova University. The growing media consisted of 38 % peat, 25 % volcanic ash, 8.5 % farmyard manure and 8.5 % sand, and was fumigated with methyl bromide under plastic sheet for 10 days. Polyethylene bags (4 l) were filled with the fumigated growing media, leaving enough room from the top for seed sowing, and after placing one layer of mycorrhizal soil on the top, the germinated seeds were sown and then covered 3-5 cm with the same growing media. A 50 g mycorrhizal soil was placed into each polyethylene bag for *G. etunicatum*, *G. caledonium* and mixed inoculum treatments, and 100 g mycorrhizal soil for *G. clarum* and *G. mosseae* treatments. Thus, each inoculation treatment received an average of 1000 spores. The inoculated and non-inoculated seedlings were grown at the greenhouse under the routine cultural practices of the nursery.

Determination of infection: Plants were harvested after the leaf fall and 0.2 g root samples were stained with trypan blue in lactic acid (Koske and Gemma, 1989). The percentage of root colonization was determined with a grid line intersect method (Giovanetti and Mosse, 1980) under a microscope (40x). The percentage of root colonization was calculated as follows: 100 x colonized root number / total root number.

Growth, macronutrient and reducing sugar analyses: Plant height and stem diameter were measured in mid-September. Shoot and root dry weights were measured on dissected shoot and root parts after the leaf fall, which were oven dried at 65 ºC for 48 hr. Leaf N-P-K contents were determined at the Laboratory of Pistachio Research Institute, Gaziantep, using standard procedures. The reducing sugar contents (%) of the seedlings were determined using the Shimadzu UV-Vis spectrometer on 3 g dried stem sample according to a modified anthron method (Kaplankıran, 1992).

Statistical analyses: The experiment was conducted with 3 replications (each had 3 seedlings) in a randomized complete-block design. An analysis of variance (ANOVA) was run on the data, and Duncan Multiple Range Test was used ($p \leq 0.05$) to illustrate mean differences.

Results and Discussion

At the end of the experiment (10 months after inoculation), seedlings of three *Pistacia* species were found to be highly mycorrhizal with the inoculations (Table 1). The highest rate of inoculated roots was 96.7 % in *P. khinjuck* seedlings with *G. clarum* and *G. etunicatum*, 83.3 % in *P. vera* seedlings with *G. caledonium* and 73.3 % in *P. terebinthus seedlings* with *G. caledonium*. In *P. vera* and *P. khinjuck* seedlings, the difference between the inoculations in their ability to colonize the roots was not statistically significant. This may indicate the presence of a broad spectrum of mycorrhizal dependency in these *Pistacia* species. In *P. terebinthus* seedlings, however, mycorrhizal inoculations statistically affected the percentage of inoculated roots, and the values was lower compared to the first two *Pistacia* species, reaching the highest level with *G caledonium*. It was reported that root colonization by VAM fungi might be genetically controlled (Karagiannidis *et al.*, 1997). On the other hand, various soil properties, management practices and inoculum densities can affect the colonization rates in any experiment. Because of wide host range of VAM fungi, its specifity may be determined more by the interactions between a fungal strain and the soil than between the fungus and its host (Mosse, 1972). Our results are in accordance with the previous findings on *Pistacia* under natural inoculation (Schubert and Martinelli, 1988) and *in vitro* inoculation (Camprubi *et al.*, 1992).

It is noteworthy that the rate of inoculated roots in *Pistacia* species tested was considerably higher (up to 96.7 %) than those of previous findings in other perennial plants (Karagiannidis *et al.*, 1997; Calvet *et al.*, 2004). Inoculated seedlings can have an advantage over indigenously inoculated seedlings, since they are already colonized before planting (Lopez-Aguillon and Mosse, 1987). The production of mycorrhizal *Pistacia* rootstocks in the nursery with the selection of effective fungi may also improve transplanting success, because it was reported that mycorrhizal inoculation reduced the transplant stress in woody plants (Matosevic *et al.*, 1997). This appears to be the case for new pistachio orchards which are often established under the arid climate of the world.

Plant heights varied according to the natural growth characteristics of *Pistacia* species (Table 2). As expected, *P. vera* had the tallest seedlings, followed by *P. khinjuck* and *P. terebinthus*, respectively. Plant heights in *P. vera* seedlings were lower with most mycorrhizal inoculations, except for *G. caledonium*, than the control plants, while in *P. khinjuck* seedlings these differences were not statistically significant. In *P. terebinthus* the mycorrhizal inoculations, except for mixed inoculum, increased plant heights significantly compared to control seedlings. The mycorrhizal inoculations had no any significant effect on the stem diameters of the *Pistacia* seedlings (data not shown).

It appeared that the mycorrhizal inoculations did not increase seedling growth of *P. vera* and *P. khinjuck*, but of *P. terebinthus* for the first year in the nursery. The experimental duration might have masked the effect of mycorrhizal

Table – 2: Plant height by the mycorrhizal inoculations in *Pistacia* seedlings.

Inoculation treatments	Plant height (cm) [y]		
	P. vera	***P. khinjuk***	***P. terebinthus***
G. caledonium	62.9 ± 7.7 a [z]	41.5 ± 4.8	33.1 ± 4.2 a
G. clarum	54.5 ± 5.5 ab	38.2 ± 2.4	34.4 ± 2.7 a
G. etunicatum	48.0 ± 4.5 bc	36.2 ± 4.5	33.3 ± 2.1 a
G. mosseae	57.2 ± 5.0 ab	42.2 ± 4.0	32.5 ± 2.5 a
Mixed	40.5 ± 4.0 c	37.9 ± 4.6	24.1 ± 2.1 b
Control	61.9 ± 4.7 a	36.8 ± 3.1	26.0 ± 2.3 b

[y]: Means ± SE.
[z]: Mean separation in columns by Duncan MRT, p≤0.05.

Table – 3: Shoot dry weight by the mycorrhizal inoculations in *Pistacia* seedlings.

Inoculation treatments	Shoot dry weight (g) [y]		
	P. vera	***P. khinjuk***	***P. terebinthus***
G. caledonium	20.9 ± 10.6 a [z]	12.3 ± 1.9	9.3 ± 1.8 a
G. clarum	14.0 ± 1.9 ab	8.1 ± 0.8	7.7 ± 0.5 abc
G. etunicatum	12.9 ± 1.6 ab	7.0 ± 1.0	6.1 ± 0.5 bc
G. mosseae	11.3 ± 1.2 ab	10.7 ± 2.4	6.1 ± 0.6 bc
Mixed	6.5 ± 0.9 b	9.4 ± 2.2	5.0 ± 0.4 c
Control	18.1 ± 2.0 a	7.1 ± 1.0	8.2 ± 1.4 ab

[y]: Means ± SE.
[z]: Mean separation in columns by Duncan MRT, p≤0.05.

inoculations on seedling development in the first two *Pistacia* species. It was reported that the absence of growth enhancement in the mycorrhizal plants of *Araucaria angustifolia* was attributed to the short duration of the experiment and the small size of the plants (Muchovej *et al.*, 1992). *P. terebinthus* plants, which grow as a bush in the field, has the slowest growing habit among the *Pistacia* species (Kaska, 1990). Therefore, the effect of fungi on the seedling growth of *P. terebinthus* might have been pronounced. But, from the production standpoint, mycorrhizal inoculations did not enhance the growth of *Pistacia* seedling in the first year, which otherwise, would be beneficial to the budding process in the nursery. Shoot dry weights in *P. vera* and *P. terebinthus* seedlings were highest with *G. caledonium* inoculation when compared to control seedlings (Table 3). Although not statistically significant, shoot dry weight in *P. khinjuck* seedlings was also higher with *G. caledonium* inoculation. But, the other mycorrhizal inoculations often resulted in less shoot dry weights than the control plants. A negative effect of some mycorrhizal species on growth was reported in a previous study conducted with avocado seedlings (da Silveira *et al.*, 2002). Root dry weights of the seedlings were not statistically different with the inoculations (Table 4). Notably, the greatest values in *P. vera* was obtained with *G. caledonium,* in *P. khinjuck* with *G. mossea* and in *P. terebinthus* with mixed inoculation. The response of rootstocks to mycorrhizal inoculations were reported to vary according to genotype and mycorrhiza species in apple (Morin *et al.*, 1994), citrus (Camprubi and Calvet, 1996), grapevine (Linderman and Davis, 2001). and avocado (da Silveira *et al.*, 2002).

The leaf N concentrations in the seedlings of *P. vera* and *P. khinjuck* were significantly higher with *G. etunicatum* inoculation (Table 5). In *P. terebinthus* seedlings *G. mossea*, *G. etunicatum* and mixed inoculations significantly increased the leaf N concentrations. This result may indicate that in various *Pistacia* rootstocks a better N uptake can be obtained with certain VAM inoculations. Accordingly, many authors suggested that mycorrhizal inoculations could result in increasing absorption of N (Ikram *et al.*, 1992; Marschner and Dell, 1994). The effects of mycorrhizal inoculations on leaf P and K concentrations of the *Pistacia* species used in this study were not statistically important (data not shown). But, there are numerous studies indicating that the incorporation of mycorrhiza into fumigated or sterilized growing media improved nutrient uptakes, mainly P (Menge *et al.*, 1980; Vidal *et al.*, 1992; Morin *et al.*, 1994). In our study, the ineffectiveness of the VAM inoculations for P and K uptakes was unexpected. This can be partly explained by the relatively rich nutrient contents of the growing media used in the nursery which included peat and farmyard manure. Azcón *et al*. (2003) indicated that at the highest N and P levels in soils, mycorrhizal lettuce plants have a decreased amount of nutrients absorbed per unit area of root mass.

Inoculation with *G. caledonium* resulted in the highest reducing sugar content in the seedlings of three *Pistacia* species (Fig. 1). The positive effects of mycorrhizal inoculation on the carbohydrates status of the plants were previously reported in citrus (Nemec and Guy, 1982), grape (Shiuchien *et al.*, 1988) and avocado plants (da Silveira *et al.*, 2002). This finding may indicate the presence of a better amount of

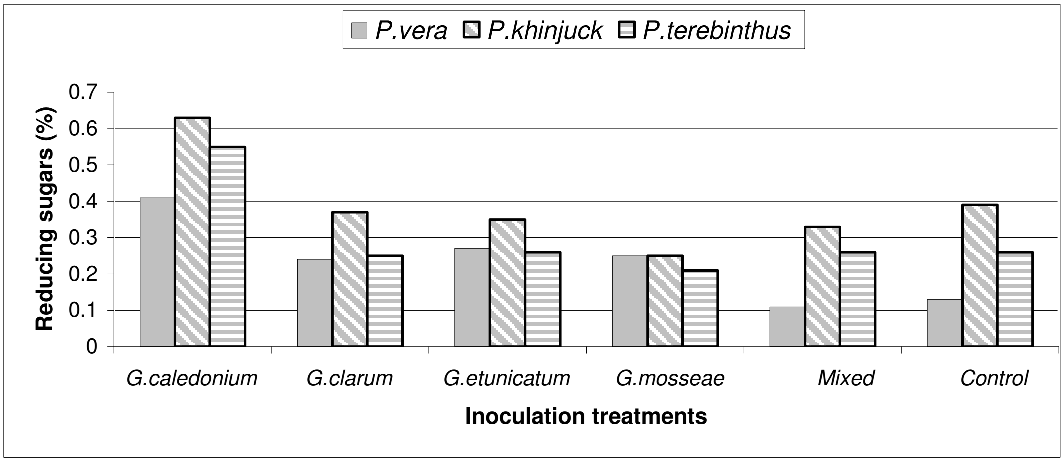

Fig. 1: Effect of mycorrhizal inoculations on the reducing sugars content in the seedlings of *P. vera, P. khinjuck* and *P. terebinthus*.

Table – 4: Root dry weight by the mycorrhizal inoculations in *Pistacia* seedlings.

Inoculation treatments	Root dry weight (g) [y]		
	P. vera	***P. khinjuk***	***P. terebinthus***
G. caledonium	12.0 ± 1.2	7.6 ± 1.0	6.7 ± 0.7
G. clarum	9.6 ± 1.2	7.2 ± 0.9	5.4 ± 0.3
G. etunicatum	11.0 ± 2.1	5.2 ± 0.8	6.6 ± 1.0
G. mosseae	9.5 ± 1.1	8.0 ± 1.1	6.2 ± 0.6
Mixed	8.8 ± 1.4	7.4 ± 0.9	7.3 ± 0.9
Control	9.9 ± 0.6	5.8 ± 0.8	6.6 ± 0.6

[y]: Means ± SE.
[z]: Mean separation in columns by Duncan MRT, $p \leq 0.05$.

Table – 5: Leaf N concentration by the mycorrhizal inoculations in *Pistacia* seedlings.

Inoculation treatments	Leaf N concentration (%)		
	P. vera	***P. khinjuk***	***P. terebinthus***
G. caledonium	0.13 b [z]	0.48 b	0.90 b
G. clarum	0.42 b	0.42 b	0.91 b
G. etunicatum	0.87 a	0.76 a	1.26 a
G. mosseae	0.60 b	0.56 b	1.22 a
Mixed	0.20 b	0.56 b	1.30 a
Control	0.47 b	0.49 b	0.97 b

[z]: Mean separation in columns by Duncan MRT, $p \leq 0.05$.

photosynthesis products with *G. caledonium* inoculation, which needs further verification. Photosynthetic rates of slow growing plants are known to be typically low (Chapin III, 1980). Therefore, *Pistacia* rootstocks which have naturally slow growing habit can benefit from the certain mycorrhizal associations in this respect.

Three *Pistacia* species used as rootstock were proved to be highly mycorrhizal with the inoculations, because the percentage of inoculated roots was considerably high, over 70 % in most cases. Incorporation of VAM fungi into the fumigated nursery growing media, practically, did not improve *Pistacia* seedling growth in the first year, which, otherwise, would be beneficial to the budding process. Certain mycorrhizal inoculations increased leaf N content of *Pistacia* seedlings, which indicates an improved N uptake in *Pistacia* species with slower growing habit than the other decidious fruit trees. The increased reducing sugar content with *G. caledonium* inoculation, in our opinion, may play a role in the second year's growth in the nursery process. In case of transplanting, pre-inoculated *Pistacia* seedlings might have a potential for a better tree growth in the harsh field conditions. In general, *G. caledonium* and *G. etunicatum* seemed to be more efficient VAM fungi in the three *Pistacia* species tested, and efforts must be made to determine the most effective VAM fungi for a particular *Pistacia* rootstock and cultivar combination under field conditions.

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