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Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake

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ABSTRACT

The influence of mycorrhiza species and growing media (GM) on the growth and nutrient uptake of citrus seedlings (sour orange (*Citrus aurantium* L.)) was studied. The experiments were conducted over 10 months using: four growth media, eight mycorrhiza species, one cocktail of mycorrhizae spores and one indigenous mycorrhiza spore (collected from the citrus orchard rhizosphere). Four different growing media were tested under controlled greenhouse conditions: GM-A, andesitic tuff + peat (1:1, v/v); GM-B, andesitic tuff + compost (1:1, v/v); GM-C, andesitic tuff + peat + compost (2:1:1, v/v/v) and GM-D andesitic tuff + peat + soil (from the Balcalı region) (2:1:1, v/v/v).

After a 10 month growing period, the highest values of leaf number, height, shoots and root dry weight were found in GM-C, followed by GM-A and GM-D; whereas the lowest values were found using the GM-B treatment. The results reveal that mycorrhizal inoculation increased the shoot and root dry weight production, compared with the levels found in non-inoculated plants. It was observed that various mycorrhiza species exhibit different responses when varying the growth media; it was also observed that *G. clarium*, *G. margarita*, *Glomus mosseae*, *Dr. Kindom* (commercial inoculum) and indigenous mycorrhiza (IM) are efficient mycorrhizae species for seedling growth. Mycorrhiza-inoculated citrus seedlings contained a higher content of phosphorus (P), zinc (Zn) than non-inoculated plants.

1. Introduction

Over 80% of the Turkish citrus plantations are located in the Eastern Mediterranean part of Turkey (Ortas, 2012). Every year, nearly one million citrus seedlings are produced in the Çukurova region for newly established citrus orchards. The soils of this part of the Çukurova region consist of high levels of clay and lime, resulting in the availability of nutrients being limited, especially phosphorus (P), zinc (Zn) and iron (Fe). In order to obtain an optimum yield, farmers use more than plant needed chemical fertilizers which cause degradation of the soil and water pollution. Accompanying the use of heavy fertilizers is a decrease in soil quality, along with the biological fertility of the soil being negatively affected. Mycorrhizae are of natural origin, and play a role in providing essential elements and water for plants, in addition to contributing to soil formation (development). It has been indicated that an arbuscular mycorrhiza fungi (AMF) association significantly increased plant height, stem diameter, leaf number per plant, shoot and root biomass, total root length, total root projected area, total root surface area and total root volume (Wu and Zou, 2010; Wu et al., 2011a). Citrus plants have a coarse and sparse root system, devoid of root hairs, making an early season nutrient and water supply crucial. A well-developed AM may increase the percentage of seedling adaptation to the field by reducing the mortality ratio. AM infection is a common association between plant roots and microorganisms; it is responsible for increasing plant nutrient uptake, especially immobile P, Zn and copper (Cu), and partly ammonium-N in soils of low fertility (Marschner, 1993; Ortas, 2003; Ortas et al., 2002a,b; Tanaka and Yano, 2005). Mycorrhizal plants normally grow more rapidly and appear healthier than non-mycorrhizal plants, especially in soils of low fertility. However, a high concentration of P was found to decrease the concentration of Zn in the tissue of the leaves and mycorrhizal activity. In addition, high levels of organic matter (Menge et al., 1982) and ammonium-N (Chambers et al., 1980; Johnson, 1984) reduce the mycorrhizal colonization of plant roots, and reduce or eliminate growth-mediated responses. Citrus rootstocks are known to have a range of dependency on mycorrhizae (Menge et al., 1978a,b; Nemec, 1979). The work of Menge et al. (1978b) demonstrated the mycorrhizal dependency of several citrus cultivars in low P soil. Citrus plants are mycorrhizal dependent (Ortas, 2012; Ortas et al., 2002b), it is therefore reasonable to produce mycorrhiza-inoculated seedlings for newly establish citrus orchards.

It has been indicated that different mycorrhizae species have shown differing responses dependent on the plant species employed (Ortas, 2010). Wu and Zou (2012), conducted

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experiments with four inoculation methods, using *Glomus mosseae* on trifoliate orange seedlings, and the results show that the method of inoculation can result in significant differences to plant growth. Nemec (1992), reported that *G. intraradix* has shown different responses to different potting materials.

Moreover, Chang and Chien (1989) have tested different mycorrhizae species on *Citrus sinensis* and found that *G. epigaeum* proved to be one of the effective mycorrhizal fungi, both in terms of mycorrhizal infection and growth response. Viyanak and Bagyaraj (1990), tested 18 different mycorrhizae species on trifoliate orange (*Poncirus trifoliate* (L.)) and found that different species show differences in plant height, stem diameter, plant biomass, P, Zn and Cu content. Khalil et al. (2011) and Cardarelli et al. (2010) reported that AM inoculated seedlings tended to increase the levels of P, K, Mg and Zn; this is significant as P and Zn deficiency is predicted extensively in the Mediterranean area, especially in citrus orchards.

Growing media also exert a significant effect on seedling growth. For the production of healthy and good quality mycorrhizae-inoculated seedlings, the selection of mycorrhizal species and growing media are very important. Furthermore, in order to achieve good growth of plants under nursery conditions, the nutrient status, organic matter content and water holding capacities are all important properties of the growing media. Ustuner et al. (2009), indicated that to derive maximal yields from organic-based farming, using AMF technology, the careful selection of an organic supplement and AMF is critical. Surprisingly, a combination of growing media is preferred to a single material (Bhagat et al., 2013). They also indicated that in the case of citrus plants to be ready for sale within one year, cocopeat and manure have the potential to improve the citrus seed germination and buddability. Schmitz et al. (2001), indicated that "soil + sand + decomposed Acacia bark" was the most appropriate substrate for the growth of *P. trifoliate*, due to its superior chemical and physical properties. However, as citrus plants respond differently to various mycorrhizae species, it is reasonable to test the effects of several mycorrhizae species and growing media on the growth and nutrient uptake of sour orange.

2. Materials and methods

2.1. Growth media and properties

One experiment was conducted, under greenhouse conditions, using the following four different substrates as a growth medium (GM):

1- GM-A: andesitic tuff + peat (1:1, v/v).
2- GM-B: andesitic tuff + compost (1:1, v/v).
3- GM-C: andesitic tuff + peat + compost (2:1:1, v/v/v).
4- GM-D: andesitic tuff + peat + soil (from the Balcalı region) (2:1:1, v/v/v).

Soil material was collected from surface horizons of the clay loam Menzilat soil series (0–20 cm) (Typic Xerofluvents) in the Çukurova Basin, which had a pH of 7.7 and the 0.5 M $NaHCO_3$ (pH 8.5) extractable P was 87.6 kg ha^{-1} P. Compost had the following characteristics: pH of 7.7, 54% organic carbon, 1.13% N, 0.18% P and 0.92% K. Peat had the following characteristics: pH of 5.1, 55% organic carbon and 0.670 EC dS m^{-1}; andesitic tuff contained 4.3% K and 0.03% P. The physical and chemical characteristics of the growth medium (soil, peat and andesitic tuff) were measured according to Page et al. (1982) in the Rhizosphere Laboratory of Çukurova University, Adana, Turkey.

2.2. Growth material sterilization

Each GM was partially sterilized in an autoclave for 2 h at 120 °C. After sterilization, the GM was left to rest for three weeks before being used for soil microbial balance.

2.3. Mycorrhizal species

Several mycorrhizal species such as *G. mosseae* (Nicolson and Gerdemann) Rothamsted isolate, UK; *G. etunicatum* (Becker and Gerdemann) Nutri-Link isolate; USA; *G. clarium* (Nicolson and Schenk) Nutri-Link isolate, USA; *G. caledonium* (Nicolson and Gerdermann) Rothamsted isolate, UK; *G. intraradices* (Smith and Schenck) and *G. margarita* (Becker and Hall) USA; *G. macrocarpum* (Tulasne and Tulasne), USA; *G. fasccículatum* (Walker and Koske) USA; *Dr. Kindom* as a commercial inoculum (which was obtained from Japan), a cocktail (a mixture of five AM species) and a mixture of indigenous mycorrhiza (isolated from the Çukurova region, Turkey) were used. Maize plants were used as the trap culture plant for mycorrhizae spore propagation.

2.4. Production of seedlings

Before sowing the seeds, the perlite was washed 2–3 times with tap water, washed once with 0.01 M HCl, rinsed twice with ionized water and then autoclaved. Mycorrhizal and non-mycorrhizal seedlings were produced under glasshouse conditions. Sour orange (*Citrus aurantium* L.) seeds were surface sterilized with a sodium hypochlorite solution (5.25%) for 2 min, rinsed several times with deionized water and then with tap water. Seeds were sown in perlite for 4–5 weeks until the seedlings reached the three-leaf stage. Subsequently, two seedlings were transplanted to a 3 kg pot.

2.5. Experimental design and growth conditions

The inoculum was calculated based on the number of spores present in 10 g of inoculum, less than 1000 spores were situated approximately 50 mm below the seedlings. In non-mycorrhizal treatments, each seedling received the same amount of mycorrhizae-free substrate (autoclaved growth medium). The experiment was completely randomized with 3 replicates.

After transplanting the seedlings, the inoculated and non-inoculated plantlets were grown for 10 months in a controlled glasshouse with day-night temperatures of 27 ± 1 °C. Plantlets were maintained with a 16 h photoperiod using cool white fluorescent lamps of 350 μ m^{-2} s^{-1}. The relative humidity was 70–80% at night and 80–85% during the day period. Distilled water was added daily to maintain the moisture at 75% of field capacity. Plants were fertilized three times with Hoagland's solution including P (11 mg kg^{-1}) and Zn (1.1 mg kg^{-1}).

2.6. Plant sampling

At the end of the growing season (10 months), shoot height (in cm), total number of leaves, shoot and root dry weight (in g $plant^{-1}$) were measured, using three samples from each treatment. At the harvest of each pot, total plant biomass (dry weight of shoot and root) and plant height were recorded. Dried material from each pot was grounded with a Tema mill, 0.2 g of the ground plant material was then ashed at 550 °C, followed by dissolution in 3.3% HCl. After digestion of the plant material, the concentration of P in this solution was determined colorimetrically (Murphy and Riley, 1962). Atomic absorption spectrophotometry was employed to determine the Zn content of the plant samples.