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Olive mycorrhization: Influences of genotype, mycorrhiza, and growing periods

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ABSTRACT

The influence of mycorrhizal inoculation using two arbuscular mycorrhizal fungal species including *Glomus mosseae* and *G. intraradices* on some growth traits and phytochemicals of olive plantlets cultivars Koroneiki and Valanolia during 20–80 weeks after inoculation was studied. The results revealed that Valanolia had higher leaf area than Koroneiki, while Koroneiki had higher height, more number of lateral shoots and leaves, higher content of phenol and higher root colonization. There was no significant difference in leaf content of total chlorophylls and carotenoids between the cultivars. The fungal species did not show any significant difference in the traits studied, except for the percentage of root colonization, which was higher in the plantlets inoculated with *G. intraradices*. However, both the arbuscular mycorrhizal treatments significantly increased the plantlets height, stem diameter, number of lateral shoots and leaves, internode length, and leaf total phenols, chlorophylls and carotenoids. In contrast, they had no significant influences on leaf area. Among the attributes measured in this experiment, internode length, leaf area, total phenols and carotenoids did not change during the growing periods, while the other traits showed an increase from 20 to 80 weeks after inoculation. The positive effects of mycorrhiza inoculation observed in the present study may be beneficial and applicable to the commercial olive nurseries.

1. Introduction

The olive (*Olea europaea* L.) is one of the oldest cultivated fruit trees and has been the main source of edible oil in the Mediterranean basin for thousands of years (Bertrand, 2002). At present, the demand for olive oil is increasing, even outside the Mediterranean basin, mainly because it has a high level of oleic acid, which is one of the healthiest fatty acids (Orlandi et al., 2003). In olive industry, propagation of semi-hardwood cuttings under mist condition has been adopted by nurseries worldwide (Fabbri et al., 2004). During vegetative propagation of olive, the number of roots initiated in each cutting influences the length of the production cycle and the quality of the rooted plantlets produced. In rooted cuttings, an important consequence of inoculation with arbuscular mycorrhizal (AM) fungi is the improvement of root system and branching and consequently the establishment of young plantlets (Hooker et al., 1992).

The AM are some associations between fungi and plant roots. They are present in the roots of almost 90% of cultivated plants and play an important role in the mineral nutrition of their hosts (Azcón-Aguilar et al., 1979). Mycorrhiza increases root surface area for water and nutrients absorption. The mycorrhizal hyphae grow from roots to soil enabling roots to contact with wider area of soil and increasing the absorbing area for water and nutrients uptake (Atkinson et al., 1994). They also have a significant role in plants protection against pathogens, increasing the resistance of plants to drought stress, and improving soil texture (Smith and Read, 1997). On the other hand, application of mycorrhizal inocula assists to increase the diversity of AM species in the rhizosphere and environment of a fruit orchard.

In recent years, there has been an increasing interest in mycorrhization of agricultural crops in order to decrease the demands for chemical fertilization. Olives have been successfully grown on marginal soils in the Mediterranean basin for thousands of years, probably due to their strong relationships with AM fungi that support them under low nutritional conditions. Accordingly, in olive plantlets, the early mycorrhizal inoculation during nursery propagation will be beneficial. Inoculation of olive plantlets with AM fungi resulted in some significant increases in their growth, precocity, production and resistance to drought and salinity (Calvente

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et al., 2004). It has been shown that AM fungi increase the rates of photosynthesis and transpiration, and stomatal conductance in wild olives (*O. europaea* ssp. *sylvestris*) (Caravaca et al., 2003). In cultivated olives, AM inoculation in the cultivar Arbequina improved plant development and production (Estaún et al., 2003) and in the cultivar 'Mission' increased root and leaf phenolic contents (Ganz et al., 2002). Different cultivars have shown different responses to the same fungal species. The inoculation of 'Moraiolo' and 'Frantoio' with *Glomus mosseae* led to an increase in plant growth, but 'Leccino' did not show any influence. Similarly, the same cultivar can respond differently to various fungal species. In Arbequina, the inoculation with *G. intraradices* showed higher rate of growth and development as compared to *G. mosseae* (Estaún et al., 2003).

The present study aimed to investigate the influence of mycorrhizal inoculation using two fungal species on some growth traits and phytochemicals of olive plantlets cultivars Koroneiki and Valanolia during 20–80 weeks after planting. The fungal strains *G. mosseae* and *G. intraradices* applied in this study are commonly exploited for mycorrhization of fruit trees.

2. Materials and methods

2.1. Plant materials

In this study, the mist propagated plantlets of two olive cultivars including Koroneiki and Valanolia were selected for inoculation. The micro-cuttings prepared to lengths of about 5 cm, leaving two pairs of leaves at the top end and rooted under mist conditions in a commercial olive nursery in Gorgan, Iran. The cultivars used in this experiment are highly favored by local growers.

2.2. AM inoculation

Two AM fungal species including *G. mosseae* and *G. intraradices*, obtained from Turan Biotech Co., Shahroud, Iran, were utilized. The inoculum contained rhizosphere soil and AM fungal spores (150/100 g dry clay soil) along with hyphae, arbuscules, and root segments of the host plant *Trifolium repens* L. Ten grams of inoculum were incorporated below the roots of the rooted cuttings during their transplantation into 0.4 L containers in mid-April. The potting mixture of soil, sand and leaf mold (2:1:1) were prepared and autoclaved (90 °C and 1.5 bar) for 30 min before inoculation. The prepared mixture had the following characteristics: K (988 mg/kg), P (180 mg/kg), Cu (0.9 mg/kg), Zn (2 mg/kg), Mn (5.2 mg/kg), Fe (6.7 mg/kg), pH 7.4 and EC 0.7 dS/m.

2.3. Growth conditions

The AM inoculated and non-inoculated control plantlets were grown in an environmentally controlled greenhouse with relative humidity maintained at 80 ± 5% and day/night temperature at 25 ± 5/12 ± 3 °C. To avoid cross contamination, each treatment group was placed on a separate block in the same greenhouse. In the first weeks, intermittent irrigation was applied to acclimatize the rooted cuttings to non-mist conditions. After that, the plantlets were irrigated to field capacity three times per week when solar radiation was less intense and once a day when the sun was at its strongest. These plantlets were kept in the same pots for one growing season and were then transplanted into 1 L containers using the same sterilized potting mixture.

2.4. Data collection

The growth and phytochemical attributes were measured 20, 40, 60 and 80 weeks after inoculation. The plants growth was determined by measuring their maximum height and the number of their leaves and lateral shoots. Stem diameter and internode length were measured between the third and sixth nodes of the new growth. Leaf areas were determined for three leaves appearing at the foregoing nodes. Leaf total phenols were estimated according to procedure suggested by Malik and Singh (1980). Leaf total chlorophylls and carotenoids were measured using the DMSO (dimethylsulphoxide) method (Barnes et al., 1992). The AM root colonization was confirmed through staining fresh root segments 80 weeks after inoculation following the procedure of Phillips and Hayman (1970).

2.5. Data analysis

The experiment was conducted as a completely randomized design with factorial arrangement including three replications each containing 10 individual plantlets. Statistical analysis was performed using GenStat (version 9) and the mean comparisons were made by the least significant difference (LSD) test. Data were suitably transformed to satisfy the assumptions of normality and constant variance prior to analysis.

3. Results and discussion

The present study showed a significant difference ($P = 0.012$) in the percentage of root colonization between the cultivars. Koroneiki (65.95%) showed more colonization as compared to Valanolia (60.37%), which correspond well with most of the growing attributes evaluated showing higher growth in Koroneiki (Tables 1 and 2). These results agree with the hypothesis that different cultivars show different responses to the same fungus (Citernesi et al., 1998; Estaún et al., 2003). Eftekhari et al. (2012) also found some differences in the percentage of root colonization among four grape cultivars. According to the results of the present study, *G. intraradices* (79.66%) showed higher percentage of root colonization ($P < 0.001$) compared to *G. mosseae* (73.33%) and non-inoculated control (22.41%). Meddad-Hamza et al. (2010) also found higher percentage of root colonization using *G. intraradices* in comparison to *G. mosseae*, though the later showed higher effects on the growing rate of the host plants. In this study, there were no differences in all the evaluated attributes between the AM fungal species. In spite of using sterile soil in this experiment, non-inoculated plants also showed some degree of colonization, which may be due to contamination with some local AM species existed in the irrigation water and greenhouse environment. Such root colonization in control plants has also observed in previous studies (Eftekhari et al., 2012; Krishna et al., 2006).

The results of this study (Table 1) showed that there was no significant difference in the plant height between the cultivars Koroneiki and Valanolia 20, 40 and 60 weeks after planting. However, 80 weeks after planting the Koroneiki plantlets (63.08 cm) grew significantly ($P < 0.001$) higher than Valanolia (55.02 cm). It seems that the growing vigor of Koroneiki became apparent in this period. In both cultivars, the height of plantlets increased significantly from 20 to 80 weeks after planting. In contrast, mycorrhizal inoculation had a significant influence on plant height in all growing periods ($P < 0.001$ in all). There was no significant difference between two fungal species, but control plantlets revealed poor growth. In all the fungal treatments, the growing periods had a significant effect on the plant height. There was no interaction between the cultivar and the fungi. In other studies, AM inoculation also increased the plant height of olive (Meddad-Hamza et al., 2010; Porras Piedra et al., 2005; Porras-Soriano et al., 2009) and in some other plants,