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Original Article

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ABSTRACT

Low and inconsistent rooting remains a major constraint to the vegetative propagation of *Olea europaea* L., owing to genotype-dependent rooting capacity and the influence of rooting substrate and beneficial micro-organisms. Therefore, this study evaluated the interactive effects of rooting substrate (perlite, cocopeat, perlite+ cocopeat), olive cultivar (Zard, Koroneiki, Roghani), and arbuscular mycorrhizal fungi (control, G. intraradices, G. mossaea, G. intraradices+ G. mossaea) on the rooting performance of olive stem cuttings under greenhouse conditions using a factorial experiment in a completely randomized design with three replications in Research Institute of Zabol on 2025. The results demonstrated that rooting performance was strongly influenced by the combined effects of cultivar, substrate, and mycorrhizal inoculation. Inoculation with Glomus intraradices consistently enhanced callus formation, rooting percentage, and the number of roots per cutting compared with the non-inoculated treatment suggesting that arbuscular mycorrhizal symbiosis promoted adventitious root formation through improved mineral nutrition, enhanced water relations, and increased root growth potential. Cocopeat promoted the highest callus formation, whereas perlite produced the greatest number of roots, indicating superior root development. Rooting responses also varied among cultivars, with the Zard cultivar exhibiting the highest root production, particularly when grown in perlite. The highest rooting percentage was achieved in the cocopeat–perlite substrate inoculated with G. intraradices. These findings demonstrate that integrating an appropriate rooting substrate with arbuscular mycorrhizal inoculation can substantially improve the vegetative propagation of olive, providing an effective strategy for producing high-quality nursery plants.

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1. Introduction

Olive (*Olea europaea* L.) is one of the world's most economically important perennial fruit crops, occupying more than 11 million hectares globally and playing a fundamental role in the agricultural economies of Mediterranean and other semi-arid regions. The increasing demand for high-quality planting material has highlighted the need for efficient vegetative propagation methods capable of producing genetically uniform and vigorous nursery plants (Petruccelli et al., 2022). However, the commercial propagation of olive by stem cuttings remains constrained by low and inconsistent adventitious root formation, which varies considerably among cultivars and is strongly influenced by propagation conditions. Successful rooting of olive cuttings depends on several interacting factors, including genotype, rooting substrate, environmental conditions, plant growth regulators, and beneficial soil microorganisms (Tanwar et al., 2013; Lanfranco et al., 2016). The presence of these organisms in diverse habitats highlights their ecological flexibility, allowing them to thrive in soils that differ in organic matter content, moisture regimes, and vegetation cover (Khosravi, 2026). Among these, arbuscular mycorrhizal fungi (AMF) have received increasing attention because they improve phosphorus acquisition, enhance water uptake, promote root growth, and improve plant tolerance to environmental stresses (Tanwar et al., 2013; Lanfranco et al., 2016). Previous studies have shown that AMF inoculation can accelerate root initiation, increase rooting percentage, improve root length, and enhance the survival and vegetative growth of olive cuttings (Ossouf et al., 2014; Semane et al., 2017; Ritter et al., 2021). Arbuscular mycorrhizal fungi (AMF) promote root development through several complementary physiological mechanisms. Beyond enhancing phosphorus uptake, AMF improve the acquisition of nitrogen and micronutrients, increase plant water-use efficiency, regulate phytohormone signaling, stimulate antioxidant activity, and facilitate carbohydrate transport to developing roots (Ladan Moghaddam & Khodami, 2017; Kaim & Bisht, 2017). Root development is essential for efficient water uptake and nutrient acquisition (Mostafavi-Fard, 2026).

Likewise, the physical characteristics of rooting substrates substantially influence aeration, water availability, and root development, resulting in considerable differences in rooting performance among substrates (Ladan Moghaddam & Khodami, 2017; Kaim & Bisht, 2017). In addition, rooting ability differs markedly among olive cultivars because of genetic variation in adventitious root formation (Younesabadi et al., 2018; Mahmoudi et al., 2025). Recent research has also demonstrated that biological inoculants and optimized propagation practices can improve rooting efficiency. For example, combinations of indole-3-butyric acid (IBA), bottom heating, and blue light significantly enhanced rooting and reduced propagation time (Asghari Gouraj, 2025), whereas beneficial microorganisms, including arbuscular mycorrhizal fungi and plant growth-promoting bacteria, improved root system development and nursery plant quality (Safari Motlagh et al., 2017; Vargas et al., 2026). The concentration of 4000 mg L^{-1} IBA was selected because previous studies have demonstrated that this concentration effectively promotes adventitious root formation and improves rooting percentage in semi-hardwood olive cuttings without causing phytotoxic effects (Hartmann et al., 2018; Mahmoudi et al., 2025).

Although previous studies have evaluated the effects of rooting substrates, olive cultivars, mycorrhizal fungi, or other biological treatments, these factors have generally been investigated independently or only in pairwise combinations. Consequently, little information is available regarding their simultaneous and interactive effects on the adventitious rooting and early root development of olive stem cuttings. Therefore, the present study was conducted to evaluate the combined effects of rooting substrate, olive cultivar, and arbuscular mycorrhizal fungi on callus formation, rooting percentage, and root development of olive stem cuttings under greenhouse conditions.

2. Material and Methods

2.1. Plant Material and Experimental Conditions

The experiment was conducted during the autumn of 2025 in the propagation greenhouse of the Agriculture Institute, Research Institute of Zabol, Iran, to evaluate the effects of rooting substrate, olive cultivar, and arbuscular mycorrhizal fungi on the rooting performance of olive (*Olea europaea* L.) stem cuttings. Healthy, vigorous, and disease-free mother plants of three olive cultivars ('Zard', 'Roghani', and 'Koroneiki') were selected as the source of propagation material. Semi-hardwood cuttings were collected from the middle and terminal portions of one- to two-year-old shoots during the early morning to minimize moisture loss. Each cutting was approximately 15 cm long and contained 4–6 healthy leaves. The basal leaves were removed from the lower one-third of each cutting to facilitate planting and hormone treatment.

2.2. Rooting Hormone Treatment

A 4000 mg L^{-1} indole-3-butyric acid (IBA) solution was prepared by first dissolving the required amount of IBA powder in a small volume of 95% (v/v) NaoH. To stimulate adventitious root formation, the basal 2–3 cm of each cutting was treated with 4000 mg L^{-1} indole-3-butyric acid (IBA). The basal ends were immersed in the IBA solution for 10 s using the quick-dip method and then immediately planted in the designated rooting medium.

2.3. Rooting Media and Mycorrhizal Inoculation

Mycorrhizal fungi contain about 100-150 spore per gram of AM species. Approximately 20 g of arbuscular mycorrhizal fungal inoculum was incorporated into each 1-L pot. The inoculum was thoroughly mixed with the upper half of the rooting substrate to ensure direct contact between the basal end of each cutting and the mycorrhizal inoculum at planting (Fig. 1). Pure perlite and cocopeat substrate poured alone into the respective pots, and a mixed substrate containing 50% perlite + 50% cocopeat was prepared based on the pot volume. All rooting media were autoclaved before use, in order to elimination of indigenous microorganisms and their effects on the experimental treatments.

2.4. Greenhouse Management

The cuttings were maintained under an intermittent mist propagation system throughout the rooting period. Bottom heating was provided to maintain a rooting-zone temperature of 24 ± 1 °C, while the greenhouse air temperature was maintained between 15 and 20 °C**.** The relative humidity inside the propagation greenhouse was maintained at 60–70% to minimize transpiration and prevent desiccation of the cuttings. The cuttings remained under these controlled environmental conditions for 60 days, after which rooting evaluations were performed. All pots were maintained under the same greenhouse conditions with uniform temperature, relative humidity, irrigation, and light throughout the experiment. Pot positions were periodically rearranged to minimize possible environmental gradients within the greenhouse.

2.5. Experimental Design

The experiment was arranged as a three-factor factorial experiment based on a completely randomized design (CRD). The experimental factors consisted of:

- Cultivar (3 levels): 'Zard', 'Roghani', and 'Koroneiki';
- Rooting medium (3 levels): perlite, cocopeat, and perlite + cocopeat (1:1, v/v);
- Mycorrhizal treatment (4 levels): control, *Glomus intraradices*, *Glomus mosseae*, and *G. intraradices* + *G. mosseae*.

The number of cuttings per replication and the total number of experimental units are 10 and 108, respectively.

Figure 1. Olive cuttings in different rooting media and mycorrhizal fungi

2.6. Data Collection

At the end of the 60-day rooting period, the following parameters were recorded: callus formation percentage, rooting percentage, number of roots per rooted cutting.

2.7. Statistical Analysis

The experimental data were subjected to analysis of variance (ANOVA) using SAS statistical software SAS 9.4. The effects of cultivar, rooting medium, mycorrhizal treatment, and their interactions were evaluated according to the factorial completely randomized design. When significant differences were detected, treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 1% probability level ($P \leq 0.01$).

3. Results

3.1. Callus Formation Percentage

The results of the analysis of variance revealed that the rooting medium and mycorrhizal fungal treatments had highly significant effects on the callus formation percentage of olive cuttings ($P \leq 0.01$). In contrast, the cultivar factor did not significantly affect callus formation. Likewise, the interactions between rooting medium and cultivar, rooting medium and mycorrhizal fungal treatment, cultivar and mycorrhizal fungal treatment, as well as the three-way interaction among rooting medium, cultivar, and mycorrhizal fungal treatment, were not statistically significant with respect to callus formation percentage. The results of cuttings rooting in 3 olive cultivars in 3 rooting media and 4 mycorrhizal fungi treatments are given in Table 1.

Table 1. Analysis of variance for rooting traits of *Olea europaea* L.

Treatments	Freedom degree	Callus percentage	Rooting percentage	Number of roots
Culture media	2	6419.7^{**}	72^{ns}	28.96^{**}
cultivar	2	92.6^{ns}	1245^{*}	4.96^{*}
Mycorrhiza fungi	3	6594.7^{**}	11862^{**}	62.95^{**}
media× cultivar	4	169.8^{ns}	1368^{**}	5.83^{**}
media× mycorrhiza	6	1275.7^{ns}	772^{ns}	2.01^{ns}
cultivar× mycorrhiza	6	709.9^{ns}	463^{ns}	1.83^{ns}
media× cultivar× mycorrhiza	12	1054.5^{ns}	669^{ns}	5.12^{**}
Error	70	66.8	63	16.7
Coefficient of Variation (CV)	-	11.7	10.36	9.63

ns is non-significant, * is significance at the 5% probability level, and ** is significance at the 1% probability level

As shown in Fig. 2, cocopeat was the most effective rooting medium for callus formation in olive cuttings, exhibiting a significantly higher callus formation percentage than both perlite and the perlite–cocopeat mixture. However, no significant difference was observed between perlite and the perlite–cocopeat medium. The difference in callus formation percentage between the best-performing medium (cocopeat) and the least effective medium was approximately 26%.

The application of mycorrhizal fungi generally increased the callus formation percentage of olive cuttings compared with the non-inoculated control treatment (Fig. 3). Among the mycorrhizal treatments, inoculation with *G. intraradices* resulted in the highest callus formation percentage and was significantly superior to all other treatments. In contrast, the treatments inoculated with *G. mosseae* and the combination of *G. intraradices* + *G. mosseae* did not differ significantly from the control treatment.

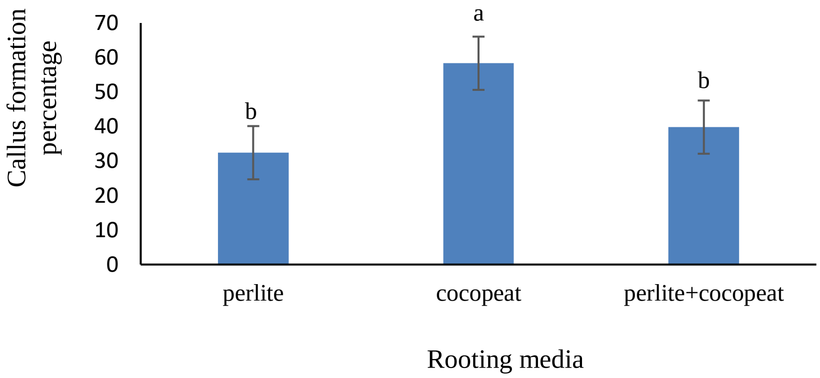

Figure 2. Effect of different rooting media on callus percentage

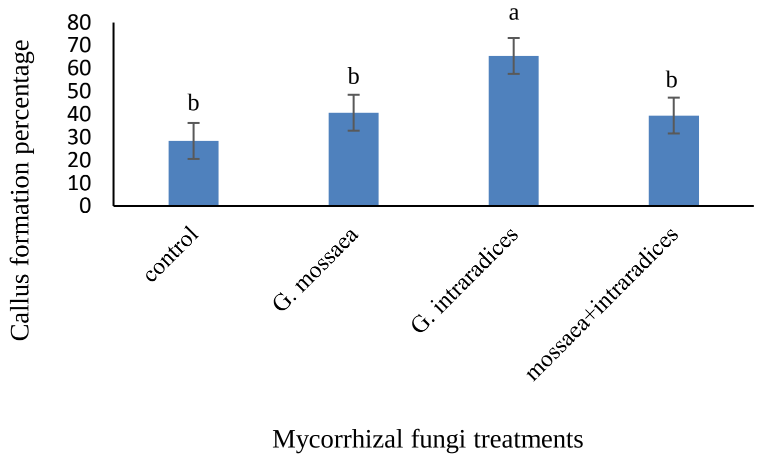

Figure 3. Effect of mycorrhizal fungi on callus percentage of olive cuttings

3.2. Rooting Percentage

Analysis of variance revealed that cultivar ($P \leq 0.05$), mycorrhizal fungal treatment ($P \leq 0.01$), and the interaction between rooting medium and cultivar ($P \leq 0.01$) significantly affected the rooting percentage of olive cuttings. However, the main effect of rooting medium and the remaining interaction effects were not statistically significant. As shown in Fig. 4, the highest rooting percentage was observed in the 'Roghani' cultivar. However, no significant difference was found between the 'Roghani' and 'Koroneiki' cultivars. Both cultivars exhibited significantly higher rooting percentages than the 'Zard' cultivar. These results indicate that rooting ability differs among olive cultivars and that genetic factors play an important role in the rooting response of cuttings.

Mycorrhizal fungal treatments significantly affected the rooting percentage of olive cuttings (Fig. 5). Among the evaluated treatments, inoculation with *G. intraradices* resulted in the highest rooting percentage, showing an approximately 50% increase compared with the non-inoculated control. All mycorrhizal treatments produced significantly higher rooting percentages than the control treatment, indicating the positive effect of mycorrhizal inoculation on adventitious root formation in olive cuttings. The combined inoculation of *G. intraradices* and *G. mosseae* resulted in a higher rooting percentage than inoculation with *G. mosseae* alone; however, its effect was lower than that obtained with *G. intraradices* alone. This finding suggests that the simultaneous application of the two fungal species did not produce a synergistic effect on rooting.

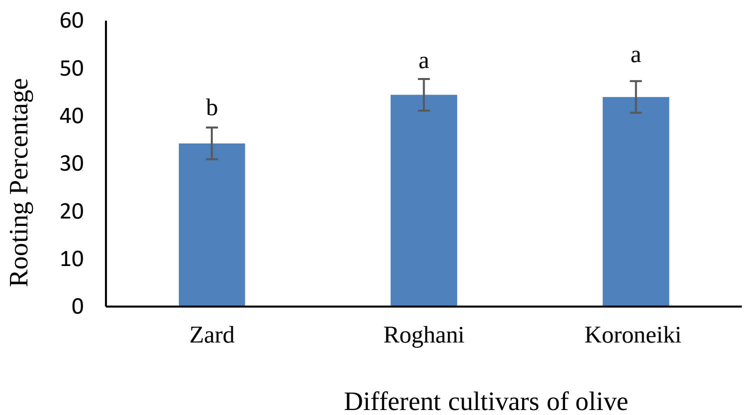

Figure 4. Effect of different cultivars on rooting percentage of olive cuttings

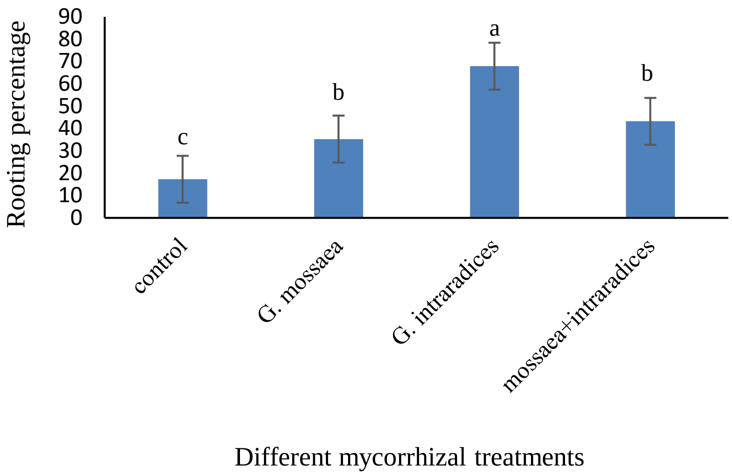

Figure 5. Effect of different mycorrhizal fungi on rooting percentage of olive cuttings

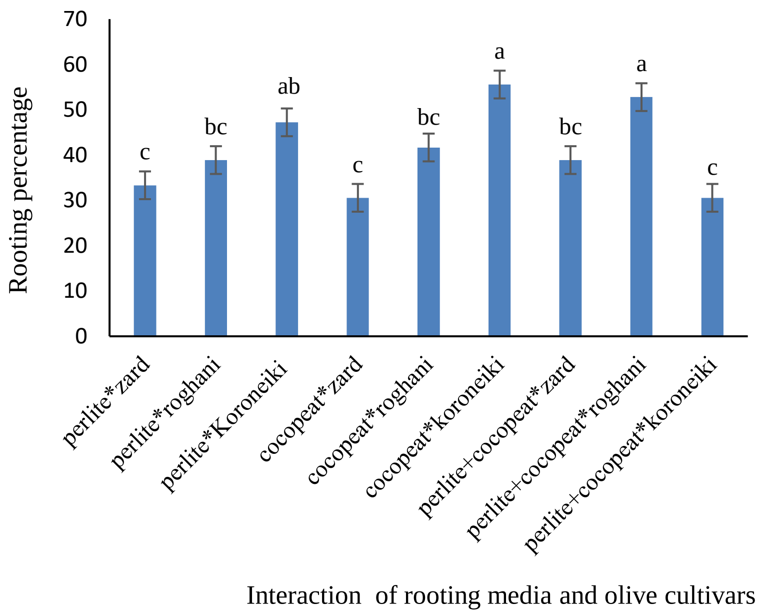

Figure 6. Interaction of media and cultivar on rooting percentage of olive

The interaction between cultivar and rooting medium had a significant effect on the rooting percentage of olive cuttings ($P \leq 0.01$) (Fig. 6). Among all treatment combinations, the highest rooting percentage was recorded for the 'Koroneiki' cultivar grown in cocopeat. However, this treatment did not differ significantly from 'Koroneiki' grown in perlite or from the 'Roghani' cultivar grown in the perlite–cocopeat mixture. The rooting percentage of the 'Zard' cultivar was not significantly affected by the type of rooting medium, as no significant differences were observed among the three media. In contrast, the 'Roghani' cultivar exhibited a significantly higher rooting percentage in the perlite–cocopeat mixture than in either perlite or cocopeat used alone. A different response was observed for the 'Koroneiki' cultivar. While this cultivar showed high rooting percentages in both pure cocopeat and pure perlite, the perlite–cocopeat mixture resulted in a reduction in rooting percentage. These findings indicate that the response of olive cuttings to rooting media is cultivar-dependent and that the optimal rooting medium varies among cultivars.

3.3. Number of Roots per Cutting

Analysis of variance revealed that rooting medium, cultivar, and mycorrhizal fungal treatment had highly significant effects on the number of roots per cutting ($P \leq 0.01$). In addition, the interaction between rooting medium and cultivar, as well as the three-way interaction among rooting medium, cultivar, and mycorrhizal fungal treatment, significantly influenced this trait at the 1% probability level.

As shown in Fig. 7, the highest number of roots per cutting was obtained in the perlite medium, although it did not differ significantly from the cocopeat medium. In contrast, the perlite–cocopeat mixture resulted in a significantly lower number of roots, indicating that the combined substrate had a negative effect on root development compared with the use of either substrate alone. Similar findings have been reported in previous studies.

The number of roots produced per cutting differed significantly among the olive cultivars (Fig. 8). The 'Koroneiki' cultivar exhibited the highest mean number of roots per cutting, although this value was not significantly different from that of the 'Zard' cultivar. In contrast, 'Koroneiki' produced significantly more roots than the 'Roghani' cultivar. These findings indicate that root production is strongly influenced by genotype, highlighting inherent differences in the rooting capacity of olive cultivars.

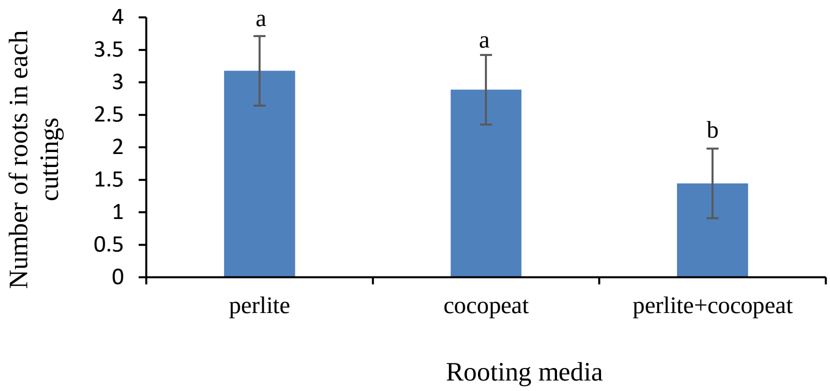

Figure 7. Effect of different media on number of roots in olive cuttings

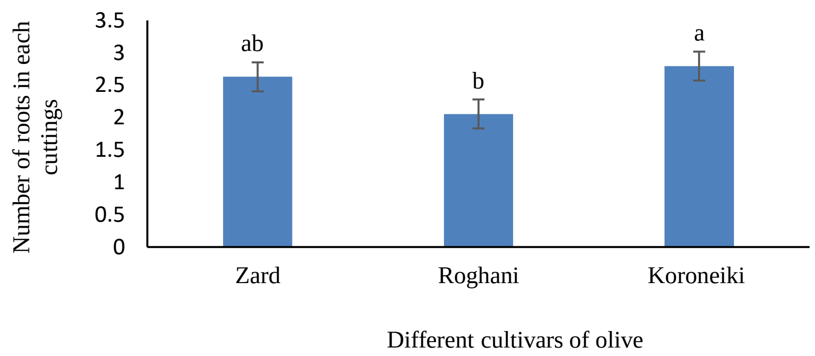

Figure 8. Effect of different cultivars on number of roots in olive cuttings

Mycorrhizal fungi significantly influenced the number of roots produced per olive cutting (Fig. 9). Cuttings grown in the medium inoculated with *Glomus intraradices* produced the highest number of roots, which was significantly greater than that observed in all other treatments. In contrast, no significant differences were detected among the control, *Glomus mosseae*, and the combined inoculation of *G. intraradices* and *G. mosseae*. These findings suggest that *G. intraradices* was more effective than the other mycorrhizal treatments in promoting root formation in olive cuttings.

The interaction between rooting medium and cultivar significantly affected the number of roots produced per cutting (Fig. 10). The highest number of roots was recorded in the 'Zard' cultivar grown in pure perlite, whereas the lowest was observed in the same cultivar grown in the perlite + cocopeat mixture. These results indicate that the rooting medium plays a more critical role in root production in some olive cultivars than in others. For the 'Zard' cultivar, no significant difference in root number was observed between the pure perlite and pure cocopeat media. In contrast, root number in the 'Roghani' and 'Koroneiki' cultivars did not differ significantly among the three rooting media. However, the perlite + cocopeat mixture consistently reduced the number of roots in all three cultivars, with the reduction being particularly pronounced in the 'Zard' cultivar compared with the pure perlite and pure cocopeat media. These findings suggest that mixed substrates may be less favorable for root initiation than single-component media, particularly for cultivars with greater sensitivity to substrate composition.

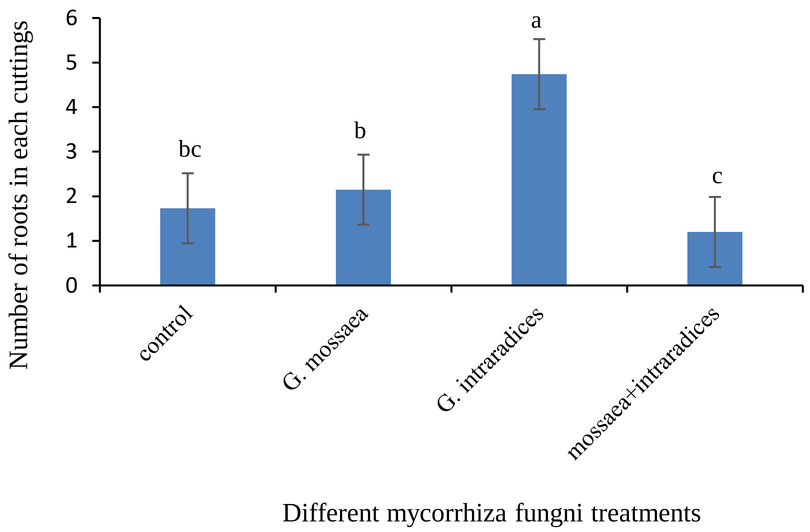

Figure 9. Effect of mycorrhizal fungi on number of roots in olive cuttings

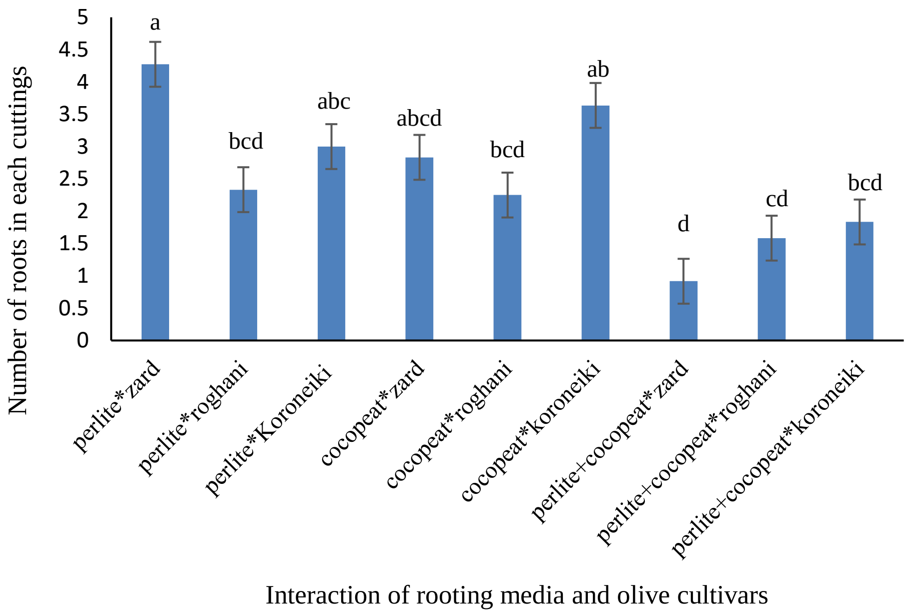

Figure 10. Interaction of media and cultivar on number of roots in olive cuttings

4. Discussion

4.1. Arbuscular mycorrhizal fungi

The present study demonstrated that inoculation with arbuscular mycorrhizal fungi (AMF), particularly *Glomus intraradices*, was associated with higher callus formation, rooting percentage, and root number in olive cuttings. These findings are consistent with those of Bidarnamani and Mohkami (2014), who reported improved rooting and root development in *Schefflera* cuttings following AMF inoculation, although combining two fungal species was less effective than the best single-species treatment. Similarly, Wu et al. (2022) showed that AMF enhanced plant growth, phosphorus uptake, and the expression of phosphate transporter genes. The positive response observed in the present study is likely associated with improved phosphorus acquisition, enhanced ATP production, and regulation of endogenous phytohormones involved in adventitious root formation. Moreover, AMF have been reported to improve nutrient and water uptake and increase antioxidant enzyme activity, thereby reducing oxidative stress during rooting (Begum et al., 2019; Chen et al., 2018; Lanfranco et al., 2018). These results support the use of *G. intraradices* as a beneficial bioinoculant for improving olive cutting propagation.

4.2. Cultivar

Significant differences in rooting among olive cultivars indicate that rooting ability is largely genotype dependent. Such variation has been attributed to differences in endogenous auxin levels, carbohydrate reserves, phenolic compounds, and the expression of genes regulating adventitious root formation (Druege, 2020). Similar genotype-dependent responses have been reported by Areek et al. (2025), who found that olive cultivars differed in rooting performance and mycorrhizal colonization following treatment with plant growth-promoting rhizobacteria and IBA. Likewise, Seifi et al. (2014) observed cultivar-dependent differences in vegetative growth, phenolic content, and AMF colonization, whereas Lopes et al. (2021) reported that mycorrhizal inoculation did not always enhance early plant growth. The superior rooting performance of the 'Zard' cultivar in the present study therefore appears to be associated with its inherent genetic capacity for adventitious root formation under the experimental conditions.

4.3. Substrate

Perlite produced the highest number of roots, whereas the cocopeat–perlite mixture resulted in poorer rooting than either substrate used individually. This unexpected response suggests that combining two suitable substrates does not necessarily provide an optimal rooting environment. Although cocopeat improves water retention and perlite enhances aeration, their combination may have altered the balance between moisture availability and air-filled porosity, thereby reducing oxygen diffusion to the basal tissues of the cuttings. Because adequate oxygen is essential for respiration, cell division, and root meristem development during adventitious rooting, suboptimal aeration may have limited root formation (Hartmann et al., 2018; Druege et al., 2019). Similar observations have been reported by Bidarnamani and Zarei (2014), who found reduced growth of pothos in a cocopeat–perlite mixture, and by Bidarnamani et al. (2014), who demonstrated that pure perlite produced superior root development in *Schefflera* and rosemary cuttings compared with cocopeat-based mixtures. Collectively, these findings indicate that the physical characteristics of the rooting substrate, particularly the balance between aeration and water availability, are more critical for successful olive propagation than the simple combination of different substrate components.

Conclusion

The present study demonstrated that rooting substrate, olive cultivar, and arbuscular mycorrhizal fungi significantly influenced the rooting performance of olive stem cuttings. Inoculation with arbuscular mycorrhizal fungi increased callus formation, rooting percentage, and root number, with *Glomus intraradices* consistently producing the most favorable responses. In contrast, the combined application of *G. intraradices* and *G. mosseae* did not provide additional benefits and, in several cases, resulted in lower rooting performance than inoculation with *G. intraradices* alone. Among the evaluated substrates, perlite produced the highest root number, while the cocopeat–perlite mixture did not improve rooting performance and generally performed less effectively than perlite alone. These findings indicate that substrate physical properties, particularly the balance between aeration and moisture availability, play a critical role in adventitious root formation. Rooting performance also differed significantly among cultivars, confirming that rooting capacity is strongly genotype dependent and that propagation protocols should be optimized for individual olive cultivars. From a practical perspective, the combination of perlite and *G. intraradices* can be recommended to improve the rooting of olive cuttings under the conditions of this study. Although rooting percentage is an important indicator of propagation success, root number provides complementary information on root system development and may be useful for evaluating the quality of rooted cuttings. However, because post-rooting growth, acclimatization, and transplant survival were not assessed, no conclusions can be drawn regarding subsequent seedling quality or field performance. Future studies should investigate whether the improved root systems observed in this study translate into greater survival and growth during nursery production and after transplanting.

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Conflict of interest

The authors have no conflicts of interest.

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