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Effects of PGPR inoculation on adventitious rooting and growth attributes in olive microcuttings of 'Mission' and 'Koroneiki' cultivars

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ABSTRACT

Purpose: This study aimed to investigate the synergistic effects of plant growth-promoting rhizobacteria (PGPR) and auxin on olive microcutting rooting, and assess how PGPR and arbuscular mycorrhizal fungi (AMF) inoculation affect the growth of rooted olive plantlets. **Research Method:** In the first experiment, native PGPR inoculation with indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) was tested to enhance rooting in olive microcuttings of 'Mission' cultivar. The second experiment evaluated six inoculation treatments (control, PGPR**,** *Funneliformis mosseae***,** *Claroideoglomus etunicatum*, PGPR + *F. mosseae*, and PGPR + *C. etunicatum*) for their impact on rooted plantlet growth of 'Mission' and 'Koroneiki' cultivars. **Findings:** The study showed that PGPR and IBA treatment for 12 weeks resulted in a higher rooting rate (63.33%) and more roots per cutting (4.5) compared to the control. Additionally, PGPR and IBA combination for 16 weeks produced the longest roots (59.03 mm), indicating PGPR's role in enhancing root initiation and growth through auxin modulation. The results also revealed that the 'Mission' cultivar had higher AMF colonization than the 'Koroneiki' cultivar. The inoculation with *F. mosseae* significantly increased the number of lateral shoots and leaves, stem diameter, and root length in 'Koroneiki', while PGPR + *F. mosseae* enhanced lateral shoots, leaf number, and stem diameter in 'Mission'. The 'Koroneiki' cultivar also exhibited greater growth responses in stem and root weights, and plant height to AMF and PGPR inoculation. **Research limitations:** No limitations were identified. **Originality/Value:** These findings underscore the importance of genetic background in biofertilization strategies for olive cultivation, demonstrating the synergistic potential of PGPR and auxin in rooting and the cultivar-specific benefits of combined PGPR and AMF inoculation.

INTRODUCTION

The olive tree (*Olea europaea* L.), native to the Mediterranean and part of the Oleaceae family, is valued for its edible fruit and oil, which are rich in monounsaturated fats and antioxidants (Visioli & Galli, 2002). However, the increasing global demand for olive products, coupled with climate challenges, is prompting changes in cultivation practices. These changes are altering traditional landscapes and potentially reshaping the structure and composition of microbial communities in orchards, which may, in turn, influence productivity and resilience to stress. The bacterial and fungal communities associated with plants are vital for plant growth and health, making them essential for sustainable agriculture (Dias et al., 2024). In particular, soil microbiota enhance plant growth, development, and overall fitness while improving soil health and fertility, which is crucial for advancing sustainable agricultural practices (Nadarajah & Abdul Rahman, 2023).

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that reside in the rhizosphere and enhance plant growth through various mechanisms. One of the primary benefits of PGPR is their ability to stimulate the production of plant hormones such as auxins, cytokinins, and gibberellins, which are pivotal for essential physiological processes, including root development and shoot elongation (Ajdig et al., 2024). Moreover, PGPR play a critical role in nutrient cycling and soil fertility by solubilizing and mobilizing essential nutrients like phosphorus and potassium, making them more accessible to plants (Maheshwari et al., 2019; Azarmi-Atajan & Sayyari-Zohan, 2020). Once established in the rhizosphere, PGPR can persist for extended periods, supported by a symbiotic relationship with the plant. Consequently, PGPR represent a valuable source of biofertilizers, biostimulants, and biocontrol agents, making them integral to organic olive production (Sallami et al., 2023).

Olive cutting propagation, a widely practiced technique, involves using stem cuttings to generate new clonal plants by relying on the innate capacity of olive tissue to form adventitious roots, often enhanced with synthetic auxins or other growth regulators (Hartmann et al., 2018). Microcutting, an alternative propagation method that uses small, excised shoot segments, offers advantages such as higher multiplication rates, better control over genetic fidelity, and the ability to propagate elite cultivars (Lambardi et al., 2023). Studies have demonstrated that PGPR can further enhance the rooting and establishment of microcuttings. For instance, Azospirillum species have been shown to stimulate root development, increase root biomass, and improve the survival rate of Prunus microcuttings (Russo et al., 2008). By leveraging the growth-promoting and stress-mitigating properties of PGPR, olive nurseries can optimize the efficiency and success of cutting propagation.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms that form mutualistic associations with the roots of many plant species, including olive trees (Palla et al., 2020). These fungi colonize the plant's root system, extending the root surface area and forming extraradical hyphae, which act as an extension of the root system (Gianinazzi et al., 2010). *Funneliformis mosseae* and *Claroideoglomus etunicatum* are two critical arbuscular mycorrhizal fungi that enhance plant growth, stress tolerance, and soil nutrient dynamics, showing promise for sustainable agriculture and ecological restoration (Berruti et al., 2016). The combined use of AMF and PGPR results in a more robust and productive plant system compared to the individual application of either microorganism (Vivas et al., 2003). The synergistic effects arise from the complementary roles of these microbes: AMF enhances nutrient and water acquisition, while PGPR stimulates plant growth and development through phytohormone production and stress alleviation. This integrated approach significantly improves olive growth (Bizos et al., 2020). Recent studies have revealed that dual inoculations of AMF and PGPR significantly improve survival, growth, physiology, and

biochemical traits of myrtle seedlings under drought stress by enhancing water and nutrient supply, stimulating antioxidant defense, and mitigating oxidative damage. This approach ultimately boosts drought tolerance and essential oil production (Azizi et al., 2021).

The aims of this study were to investigate the synergistic effects of PGPR and auxin treatments on the adventitious rooting in olive microcuttings of the 'Mission' cultivar and to examine the influence of PGPR and AMF inoculation on the growth attributes of rooted olive plantlets in the 'Mission' and 'Koroneiki' cultivars. The 'Mission' and 'Koroneiki' are two significant olive cultivars well-suited to the climatic conditions of the research area. Each possesses distinct characteristics and applications. The 'Mission' cultivar is versatile, suitable for both table olives and oil production, whereas the 'Koroneiki' is renowned for its robust, high-quality oil, characterized by a peppery and aromatic flavor.

MATERIALS AND METHODS

This research involved two experiments designed to investigate the effect of inoculation with native PGPR on the rooting of olive microcuttings and the growth of the rooted young plantlets. The experiments were conducted in 2020 in the greenhouse and laboratory of the Department of Horticultural Sciences at Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Isolation and preparation of PGPR inoculum

To isolate PGPR, four soil samples were collected from the rhizosphere of olive trees in a standard orchard, adhering to biological sampling principles. Ten grams of rhizosphere soil were mixed with 90 mL of physiological serum (0.9% sodium chloride) and shaken for 30 minutes at 150 rpm. Serial dilutions were prepared, and two replicates of samples from five lower dilutions (108, 107, 106, 105 and 104) were plated on nutrient agar medium. After bacterial growth, 24 bacterial isolates were selected based on colony shape, morphology, and color and combined to prepare the inoculum (Fig. 1A) (Marzban et al., 2019).

Experiment 1: The effect of PGPR inoculation on olive microcuttings rooting

This experiment followed a factorial design based on a completely randomized design with three replications, each comprising 10 microcuttings. The first factor was the rooting period, with two levels (12 and 16 weeks). The second factor included a non-inoculated control and PGPR inoculation, while the third factor consisted of treatments with indole-3-butyric acid (IBA) (3 g/L), naphthaleneacetic acid (NAA) (1.5 g/L), and controls. Microcuttings were prepared from healthy, mature 'Mission' cultivar trees, aged 8 years, grown in a standard orchard. Current-year shoots were harvested in the first week of October, and microcuttings (4-6 mm in diameter, 10-12 cm in length) were prepared, treated, and planted at a depth of 3-4 cm in rooting medium within greenhouse cultivation trays equipped with a misting system (Fig. 1B).

The culture medium consisted of perlite with a particle diameter of 1.5 to 2.5 mm, sterilized in an autoclave at 121°C and 15 psi for 15-20 minutes. Sterile perlite was used for the non-inoculated control, while perlite containing 107 CFU/mL bacteria was applied for PGPR inoculation (Liffourrena & Lucchesi, 2018). Microcuttings were treated with auxin for 10 seconds, while control microcuttings were treated with distilled water. After 12 and 16 weeks, the parameters measured included rooting percentage, number of roots, average root length, fresh root weight, and dry root weight.

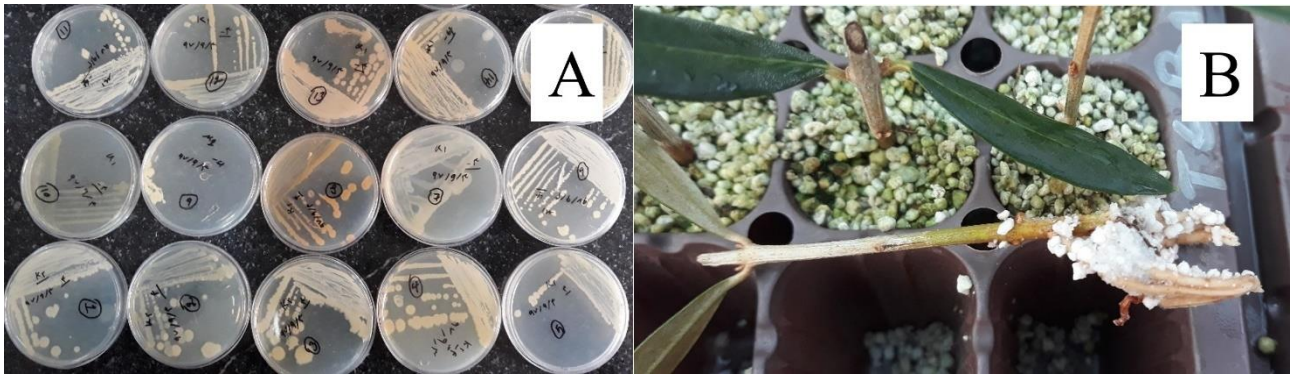

Fig. 1. A) Isolation and preparation of plant growth-promoting rhizobacteria; B) rooted microcuttings.

Experiment 2: The effect of PGPR and AMF inoculation on the growth of rooted olive plantlets

This experiment employed a factorial design based on a completely randomized design. The first factor was the cultivar, with two levels ('Koroneiki' and 'Mission'), and the second factor was inoculation with six treatments: non-inoculated control, PGPR, *F. mosseae*, *C. etunicatum*, PGPR + *F. mosseae*, and PGPR + *C. etunicatum*, with seven replications. Plantlets were planted in 2 L pots filled with a 1:1:1 mixture of agricultural soil, vermicompost, and sand. The potting mixture had the following composition: C (1.4%), N (0.10%), K (368 mg/kg), P (10.2 mg/kg), pH 7.7, and EC 1.5 dS/m. The mixture was sterilized at 121°C and 1.5 bars for one hour. Plantlets were grown in a controlled greenhouse environment with a relative humidity of 75 ± 10% and a daytime temperature of 25 ± 5°C.

For PGPR inoculum preparation, a bacterial population was added to NB medium at 2% volume and incubated for 48 hours on a shaker at 150 rpm. Plantlet roots were soaked in the PGPR suspension for 30 minutes prior to planting. AMF (*F. mosseae* and *C. etunicatum*) inoculum, obtained from Toran Biotechnology Company, consisted of rhizosphere soil, AMF spores (minimum 30 spores/g soil), hyphae, arbuscules, and root segments of *Trifolium repens* L. Each pot was inoculated with 40 g of fungi powder mixed into the potting medium. For combined PGPR and AMF treatments, roots were first soaked in the PGPR inoculum, followed by the application of AMF inoculum in the rhizosphere.

At the end of the seven-month growing period, the plant characteristics measured included the number of new lateral branches and leaves, leaf area per plant, internode length, plant height, stem diameter, and root length. Root colonization by symbiotic structures was determined 12 weeks post-inoculation via root staining and microscopic examination (Dalpe, 1993). Fresh weights of stems and roots were recorded, followed by dry weight determination after drying at 70°C for 48 hours (Ganjeali & Kafi, 2007). Total chlorophyll, chlorophyll a, and chlorophyll b content in leaves were measured using the DMSO method (Barnes et al., 1992), and total phenolic content was quantified using the Folin-Ciocalteu method and a spectrophotometer at 760 nm (Seifi & Bekran, 2024).

Data analysis

All data were analyzed using SAS software (version 9.3). Means were compared using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The effects of PGPR inoculation on adventitious rooting

The analysis of variance revealed a significant interaction effect between rooting period, PGPR inoculation, and auxin treatment on rooting percentage (P<0.001), root number (P=0.004), and root length (P<0.001) (Table 1). These findings align with previous studies that have demonstrated the synergistic effects of PGPR and auxin on root development in various plant species (Vacheron et al., 2013; Maniriho et al., 2021). Specifically, the data indicated that PGPR inoculation combined with IBA after 12 weeks resulted in a significantly higher rooting rate (63.33%) and more roots per cutting (4.5) compared to the control, which showed a rooting rate of only 3.33% and 0.33 roots per cutting. This supports the literature suggesting that PGPR enhances root initiation and growth by modulating auxin signaling and metabolism (Spaepen et al., 2007). Moreover, PGPR inoculation with IBA after 16 weeks produced the longest root length, reaching 59.03 mm. This result underscores the role of PGPR in promoting root elongation by solubilizing nutrients and making them available for plant growth and development (Russo et al., 2008; Vacheron et al., 2013).

Table 1. The interaction and independent effects of rooting period, PGPR inoculation, and auxin treatment on rooting percentage and root characteristics in olive cultivar 'Mission'.

Rooting period	PGPR	Auxin	Rooting (%)	Root (n)	Root length (mm)	Root fresh weight (g)	Root dry weight (g)
			P<0.001	P=0.004	P<0.001	P<0.001	P=0.223
12 wk	Non-inoculated	Control	3.33 f	0.33 f	14.33 g	0.03 e	0.006
		IBA	33.33 c	4.43 a	32.16 f	0.21 a	0.015
		NAA	16.63 e	3.16 c	44.83 b	0.20 a	0.025
	Inoculated	Control	0.00 f	0.00 f	0.00 i	0.00 g	0.000
		IBA	63.33 a	4.50 a	36.20 d	0.18 b	0.019
		NAA	20.00 d	2.60 d	33.93 e	0.11 d	0.026
16 wk	Non-inoculated	Control	20.00 d	0.83 e	4.61 h	0.02 ef	0.007
		IBA	60.00 b	2.29 d	45.27 b	0.13 c	0.021
		NAA	33.33 c	4.36 ab	36.20 d	0.13 c	0.020
	Inoculated	Control	20.00 d	1.21 e	13.90 g	0.01 fg	0.011
		IBA	60.00 b	4.36 ab	59.03 a	0.20 a	0.028
		NAA	33.33 c	4.00 b	40.52 c	0.20 a	0.027
Rooting period			P<0.001	P<0.001	P<0.001	P=0.816	P=0.659
12 wk			22.77 b	2.50 b	26.91 b	0.12	0.015
16 wk			37.77 a	2.84 a	33.25 a	0.11	0.019
PGPR			P<0.001	P=0.015	P<0.001	P=0.387	P=0.248
Non-inoculated			27.77 b	2.57 b	29.56 b	0.12	0.015
Inoculated			32.77 a	2.78 a	30.59 a	0.11	0.018
Auxin			P<0.001	P<0.001	P<0.001	P=0.035	P=0.820
Control			10.83 c	0.59 c	8.21 c	0.02 c	0.006
IBA			54.16 a	3.90 a	43.16 a	0.18 a	0.021
NAA			25.82 b	3.53 b	38.87 b	0.16 b	0.024

Different letters in each column represent significant differences at P=0.01, Duncan's multiple range test. The abbreviations are as following: IBA (indole-3-butyric acid), NAA (naphthaleneacetic acid), and PGPR (plant growth-promoting rhizobacteria).

The analysis of variance also showed a significant interaction effect of the three treatments on root fresh weight (P<0.001) but not on root dry weight (P=0.223) (Table 1). This suggests that the treatments primarily influenced water content and cellular expansion in the roots rather than overall biomass accumulation (Glick, 2012). The highest root fresh weight recorded was 0.21 g after treatment with IBA for 12 weeks without PGPR inoculation, indicating that IBA treatment alone had a more pronounced effect on root fresh weight. The lack of a significant effect on root dry weight suggests that none of the treatments directly contributed to substantial biomass accumulation, implying that water retention and expansion, rather than an increase in solid biomass, were the primary drivers of the observed treatment effects.

The independent effects of rooting period, PGPR inoculation, and auxin treatment on rooting percentage, root number, and root length were significant (Table 1). This highlights the critical role of these individual factors in promoting root development in the studied plant system (Hartmann et al., 2018). Notably, the data show that PGPR inoculation and auxin application, particularly IBA, significantly increased all three root traits. PGPR inoculation enhanced rooting significantly, achieving 32.77% compared to 22.77% in the non-inoculated control. Among auxin treatments, IBA induced the highest rooting rate at 54.16%, significantly outperforming the control (10.83%) and NAA (25.82%). A similar trend was observed for root length, with IBA-treated cuttings reaching 43.16 mm, compared to 38.87 mm for NAA and 8.21 mm for the control. These findings are consistent with previous research demonstrating the synergistic effects of PGPR and auxin on root growth and development (Vacheron et al., 2013). In contrast, the independent effects of the three treatments on root fresh weight and root dry weight were not significant, except for the effect of auxin on root fresh weight, which was significant (P=0.035). The superior efficacy of IBA over NAA in promoting root development aligns with prior studies highlighting IBA's greater effectiveness in stimulating root formation and growth (Pacurar et al., 2014). This difference may be attributed to the distinct mechanisms of action and transport dynamics of these two auxin compounds within plant tissues.

The effects of PGPR and AMF inoculation on growth attribute

The results of this study indicate that olive plantlets of the 'Mission' cultivar exhibited a higher percentage of colonization by AMF compared to the 'Koroneiki' cultivar (Fig. 2). In the 'Mission' cultivar, the highest colonization percentage was recorded at 89.19%, significantly higher than the 22.27% observed in the control treatment. This suggests that the 'Mission' cultivar is more responsive to AMF inoculation, potentially due to its genetic makeup or physiological characteristics. These findings align with the hypothesis proposed by Estaún et al. (2003), which suggests that different cultivars exhibit varying responses to the same fungal species. Similarly, Eftekhari et al. (2012) reported differences in root colonization percentages across four grape cultivars. Interestingly, within the 'Mission' cultivar, no significant differences were observed among the other main treatments of AMF or the combined treatments of PGPR with AMF. This indicates that combined inoculation did not have a synergistic effect on colonization percentage in this cultivar. The lack of a significant interaction between PGPR and AMF suggests that AMF inoculation alone may be sufficient to achieve optimal colonization levels in the 'Mission' cultivar. In contrast, the 'Koroneiki' cultivar exhibited significant differences in colonization percentages among the AMF treatments. The highest colonization was observed with *F. mosseae* at 76.5%, while the lowest was recorded with the PGPR + *C. etunicatum* treatment at 63.47%. These results suggest that cultivars may have varying sensitivities to different AMF species, a phenomenon also reported by Berruti et al. (2016). Additionally, findings by Seifi et al. (2014) reported

that *Glomus intraradices* achieved a higher colonization percentage (79.66%) in olive plantlets compared to *G. mosseae* (73.33%). The low levels of contamination by native fungi observed in the control treatments did not appear to affect the growth of the olive plantlets, consistent with results from other studies (Eftekhari et al., 2012; Ziatabar Ahmadi et al., 2024). This suggests that native fungal communities may not significantly impact the plant's performance, and the observed differences in colonization percentages can be attributed to the inoculated treatments.

The study's results indicate that inoculation with *F. mosseae* had the most significant positive effect on the number of new lateral shoots in the 'Koroneiki' olive cultivar, with the highest number recorded at 4 (Table 2). Conversely, the 'Mission' cultivar exhibited the highest number of new lateral shoots (4) when inoculated with PGPR + *C. etunicatum* (Fig. 3). This suggests that, for the 'Mission' cultivar, a synergistic interaction between PGPR and AMF may be more effective in stimulating lateral shoot production than either inoculant alone. These findings highlight the cultivar-specific nature of microbial interactions and the importance of tailoring inoculation strategies to the genetic and physiological characteristics of each olive cultivar. The lowest number of new lateral shoots (1.3) was observed in the control treatment of the 'Koroneiki' cultivar, suggesting that inoculation with beneficial microorganisms can enhance the production of new lateral shoots in olive plants (Hanane et al., 2020).

The highest number of new leaves (112) was recorded in the *F. mosseae* treatment (Table 2), demonstrating the positive impact of this AMF on leaf production (Russo et al., 2008; Meddich et al., 2015). Similarly, Chenchouni et al. (2020) reported that AMF application improved the number of leaves in olive plantlets. Conversely, the control treatments exhibited the fewest leaves, averaging just 10 and 18 in the 'Koroneiki' and 'Mission' cultivars, respectively. This highlights the importance of inoculating olive plantlets with beneficial soil microorganisms to promote vegetative growth.

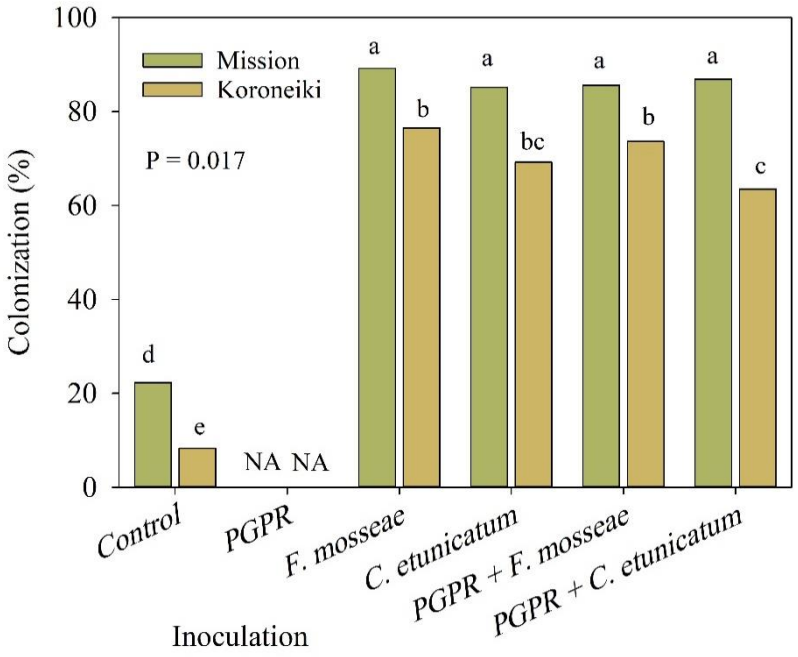

Fig. 2. The colonization percentage of different treatments in olive plantlets of cultivars 'Mission' and 'Koroneiki'. Different letters represent significant differences at P=0.05, Duncan's multiple range test. The abbreviations are as following: NA (not applicable), and PGPR (plant growth-promoting rhizobacteria).

Table 2. The interaction effects of PGPR and AMF inoculation on some growth parameters of olive plantlets cultivars 'Mission' and 'Koroneiki'.

Cultivar	Inoculation	New shoot (n)	New leaves (n)	Leaf area/ plant (cm^2)	Internode length (cm)	Plant height (cm)	Stem diameter (mm)	Root length (cm)
		P<0.001	P<0.001	P<0.001	P=0.002	P<0.001	P=0.031	P=0.022
Mission	Control	2.0 d	18.00 f	29.42 ef	0.86 f	13.5 e	2.74 d	25.66 efg
	PGPR	2.0 d	24.33 e	77.96 e	1.40 e	14.83 e	2.64 d	29.33 cde
	F. mosseae	3.0 bc	51.33 c	263.54 ab	2.33 abc	33.16 c	3.29 cd	26.56 d-g
	C. etunicatum	3.3 b	38.66 d	148.94 d	2.05 cd	24.26 d	2.72 d	25.26 fg
	PGPR + *F. mosseae*	4.0 a	69.00 b	157.57 cd	1.85 d	25.33 d	3.72 b	24.26 g
	PGPR + *C. etunicatum*	3.0 bc	47.66 c	168.62 cd	1.80 d	25.26 d	2.92 cd	24.16 g
Koroneiki	Control	1.3 e	10.33 g	14.71 f	0.46 g	5.66 f	2.71 d	32.66 bc
	PGPR	2.7 c	19.33 ef	38.58 ef	1.16 ef	10.93 e	2.91 cd	38.83 a
	F. mosseae	4.0 a	112.00 a	279.35 a	2.11 bcd	49.10 a	4.25 a	39.50 a
	C. etunicatum	2.0 d	64.33 b	213.19 bc	2.53 a	45.36 a	3.45 bc	35.03 b
	PGPR + *F. mosseae*	3.0 bc	65.33 b	319.57 a	1.96 cd	38.43 b	3.69 b	28.23 def
	PGPR + *C. etunicatum*	3.0 bc	40.66 d	184.48 cd	2.45 ab	32.90 c	3.64 b	29.50 cd

Different letters in each column represent significant differences at P=0.01, Duncan's multiple range test. The abbreviations are as following: AMF (arbuscular mycorrhizal fungi) and PGPR (plant growth-promoting rhizobacteria).

Fig. 3. The effects of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi inoculation on growth of olive plantlets cultivar 'Mission'. 1: Control, 2: PGPR, 3: *F. mosseae*, 4: *C. etunicatum*, 5: PGPR + *F. mosseae*, 6: PGPR + *C. etunicatum*.

The results also demonstrate that the highest leaf area per plant was observed in the 'Koroneiki' cultivar, with the *F. mosseae* and PGPR + *C. etunicatum* treatments recording 279.35 cm^2 and 319.57 cm^2, respectively (Table 2). In the 'Mission' cultivar, the highest leaf area per plant was recorded in the *F. mosseae* treatment at 263.54 cm^2. These findings align with those of Meddich et al. (2015), who reported significant improvements in leaf area in palm seedlings inoculated with *G. monosporus* and *G. clarum* compared to control plants. Similarly, Khalil and El-Ansary (2020) reported that 'Manzanillo' olive plantlets inoculated with AMF exhibited significantly higher leaf areas than non-inoculated control plantlets. Interestingly, the effect of the PGPR treatment on leaf area was more pronounced in the 'Mission' cultivar than in the 'Koroneiki' cultivar. This indicates cultivar-specific responses of

olive plantlets to beneficial microorganism inoculation, highlighting the importance of considering the genetic background and physiological characteristics of different plants when implementing biofertilization strategies (Berruti et al., 2016).

In the 'Koroneiki' cultivar, inoculation with the AMF species *C. etunicatum* resulted in the highest internode length of 2.53 cm. In contrast, the shortest internode lengths were observed in the control treatments, measuring 0.46 cm (Table 2). This finding is consistent with previous research demonstrating the ability of AMF to enhance nutrient uptake and improve plant growth characteristics, such as internode length (Seifi et al., 2014; Berruti et al., 2016). Additionally, PGPR application increased internode length in both cultivars compared to the control. PGPR is known to produce growth-promoting substances, such as auxins, cytokinins, and gibberellins, which stimulate cell elongation and division, thereby enhancing plant growth parameters (Glick, 2012).

The highest plant heights of 49.10 cm and 45.36 cm were achieved with *F. mosseae* and *C. etunicatum* inoculation, respectively, in the 'Koroneiki' cultivar (Table 2), further supporting the positive effects of AMF on plant growth (Berruti et al., 2016). Interestingly, no significant difference in plant height was observed between PGPR-inoculated (14.83 cm) and control plants (13.5 cm) in the 'Mission' cultivar. However, in the 'Koroneiki' cultivar, PGPR inoculation increased plant height to 10.93 cm compared to 5.66 cm in the control, indicating that the response to PGPR may be cultivar-dependent. Research has shown that AMF inoculation can significantly improve plant growth parameters. For instance, Chenchouni et al. (2020) reported that AMF inoculation increased plant heights in olive plantlets, with native AMF species particularly effective. They observed plant heights ranging from 77.2 cm in *Glomus* sp.2-inoculated plants to 145.6 cm in *Glomus* sp.1-inoculated plants, compared to an average height of 54.6 cm in control plants.

The highest root lengths were observed in the 'Koroneiki' cultivar, with *F. mosseae* and PGPR inoculation yielding root lengths of 39.50 cm and 38.83 cm, respectively (Table 2). The treatments' effect on increasing root length was more pronounced in the 'Koroneiki' cultivar than in the 'Mission' cultivar. The highest root length in 'Mission', measuring 29.33 cm, was observed with PGPR inoculation. This finding aligns with Hanane et al. (2020), who reported that olive young plants inoculated with the *Rhizolive consortium* or *G. irregulare* had greater root lengths compared to control plants. Similarly, Hadjouti et al. (2022) demonstrated that certain PGPR species significantly increased root length in zucchini compared to control.

The study also revealed that the highest stem fresh weight of 3.78 g was recorded in the 'Koroneiki' cultivar treated with *F. mosseae* (Table 3). Extensive research supports that AMF inoculation can significantly enhance plant growth and development across various species (Berruti et al., 2016; Chenchouni et al., 2020). Additionally, PGPR significantly increased stem fresh weight in both 'Koroneiki' and 'Mission' cultivars compared to the control. By producing growth-promoting substances like auxins, cytokinins, and gibberellins, PGPR stimulate cell growth and division, leading to improved plant biomass (Glick, 2012). The highest stem dry weight of 1.89 g was observed in the 'Koroneiki' cultivar treated with *F. mosseae*, over seven times higher than the control (0.25 g). This finding is consistent with previous research demonstrating AMF's ability to enhance nutrient uptake and biomass production (Rodrigues et al., 2021). Similarly, PGPR inoculation significantly increased stem dry weight in both cultivars compared to the control. These findings suggest that applying AMF and PGPR enhances both fresh and dry plant weights, reflecting overall improved growth and development. By producing growth-regulating substances such as auxins, cytokinins, and gibberellins, AMF and PGPR promote cellular growth and division, resulting in increased biomass accumulation.

Table 3. The interaction effects of PGPR and AMF inoculation on fresh and dry weight of stem and root in olive plantlets of cultivars 'Mission' and 'Koroneiki'.

Cultivar	Inoculation	Stem fresh weight (g)	Stem dry weight (g)	Root fresh weight (g)	Root dry weight (g)
		P<0.001	P<0.001	P<0.001	P<0.001
Mission	Control	0.42 g	0.26 e	0.28 d	0.23 d
	PGPR	0.92 ef	0.58 d	0.34 d	0.24 d
	F. mosseae	1.93 bc	1.18 b	0.27 d	0.21 d
	C. etunicatum	1.04 e	0.65 d	0.41 cd	0.33 bcd
	PGPR + *F. mosseae*	1.18 de	0.68 d	0.34 d	0.26 d
	PGPR + *C. etunicatum*	1.60 cd	1.10 cd	0.26 d	0.21 d
Koroneiki	Control	0.50 fg	0.25 e	0.27 d	0.23 d
	PGPR	1.19 de	0.71 d	0.53 c	0.41 bc
	F. mosseae	3.78 a	1.89 a	1.27 a	0.82 a
	C. etunicatum	2.12 b	1.26 b	0.54 c	0.43 b
	PGPR + *F. mosseae*	2.13 b	1.12 bc	0.97 b	0.72 a
	PGPR + *C. etunicatum*	1.51 cd	0.85 cd	0.36 d	0.27 cd

Different letters in each column represent significant differences at P=0.01, Duncan's multiple range test. The abbreviations are as following: AMF (arbuscular mycorrhizal fungi) and PGPR (plant growth-promoting rhizobacteria).

This study also examined the effects of AMF and PGPR on root growth. The highest root fresh weight of 1.27 g was observed in the 'Koroneiki' cultivar after inoculation with *F. mosseae* (Table 3). In this cultivar, most treatments caused a significant increase in root fresh weight, whereas in the 'Mission' cultivar, all treatments had no significant effect on root fresh weight. Similar results were reported by Chenchouni et al. (2020), who found that native AMF species, particularly Glomus sp.1, produced the highest root fresh weight in olive plantlets compared to control plants. The highest root dry weight in the 'Koroneiki' cultivar was 0.82 g after inoculation with *F. mosseae* and 0.72 g after inoculation with PGPR + *F. mosseae*. In contrast, treatments had no significant effect on root dry weight in the 'Mission' cultivar. These findings suggest that the 'Koroneiki' cultivar may be more responsive to AMF and PGPR applications compared to the 'Mission' cultivar, highlighting the importance of considering cultivar-specific responses in olive cultivation. Mycorrhizal fungi, such as *F. mosseae*, enhance water and nutrient uptake through symbiotic relationships with plant roots, leading to improved biomass production (Rodrigues et al., 2021).

The study also revealed that the highest total chlorophyll content was observed in the control treatment and the PGPR + *C. etunicatum* treatment (1.86 and 1.73 mg/g FW, respectively), followed by the control and PGPR treatments of the 'Koroneiki' cultivar (Fig. 4A). Conversely, the lowest total chlorophyll content was observed with the inoculation of *C. etunicatum* in the 'Mission' cultivar (0.87 mg/g FW) and *F. mosseae* in the 'Koroneiki' cultivar (0.83 mg/g FW). This contrasts with findings by Esna-Ashari and Bahrami (2018), who reported increased chlorophyll a, b, and total chlorophyll content with AMF inoculation compared to controls. The discrepancy may arise from specific interactions between plant cultivars, microbial strains, and environmental conditions (Seifi et al., 2014). The highest chlorophyll b content was observed in the control and PGPR + *C. etunicatum* treatments of the 'Mission' cultivar (1.15 and 0.99 mg/g FW, respectively) (Fig. 4B). In contrast, the lowest chlorophyll b content was recorded in the *F. mosseae* treatment of the 'Mission' cultivar. This suggests that *F. mosseae* inoculation may negatively impact chlorophyll b content in the 'Mission' cultivar, potentially affecting photosynthetic performance. The analysis of variance revealed no significant differences in chlorophyll a content between the inoculation treatments or cultivars studied (Fig. 4C and D), suggesting that cultivar differences and microbial

inoculations had a more pronounced effect on chlorophyll b and total chlorophyll content rather than chlorophyll a content. These findings reveal that while AMF and PGPR applications significantly boosted olive plant growth, they were associated with a decrease in chlorophyll content. This could be due to resource allocation, as AMF and PGPR enhance nutrient uptake (Berruti et al., 2016). Additionally, this pattern may reflect a shift in physiological priorities, where the growth enhancement driven by AMF and PGPR leads to a dilution effect in chlorophyll concentration due to increased biomass production. These findings underscore the intricate relationship between microbial inoculations, photosynthetic pigment dynamics, and overall plant development, emphasizing the need for further research to elucidate the underlying mechanisms. This paradox underscores the complex interactions between plant growth, nutrient uptake, and physiological responses. Additionally, the lack of stress in the plants used in this experiment may explain the discrepancies. Ye et al. (2022) demonstrated that under water stress conditions, AMF inoculation significantly increased chlorophyll b and total chlorophyll content, whereas no such effect was observed under non-stress conditions.

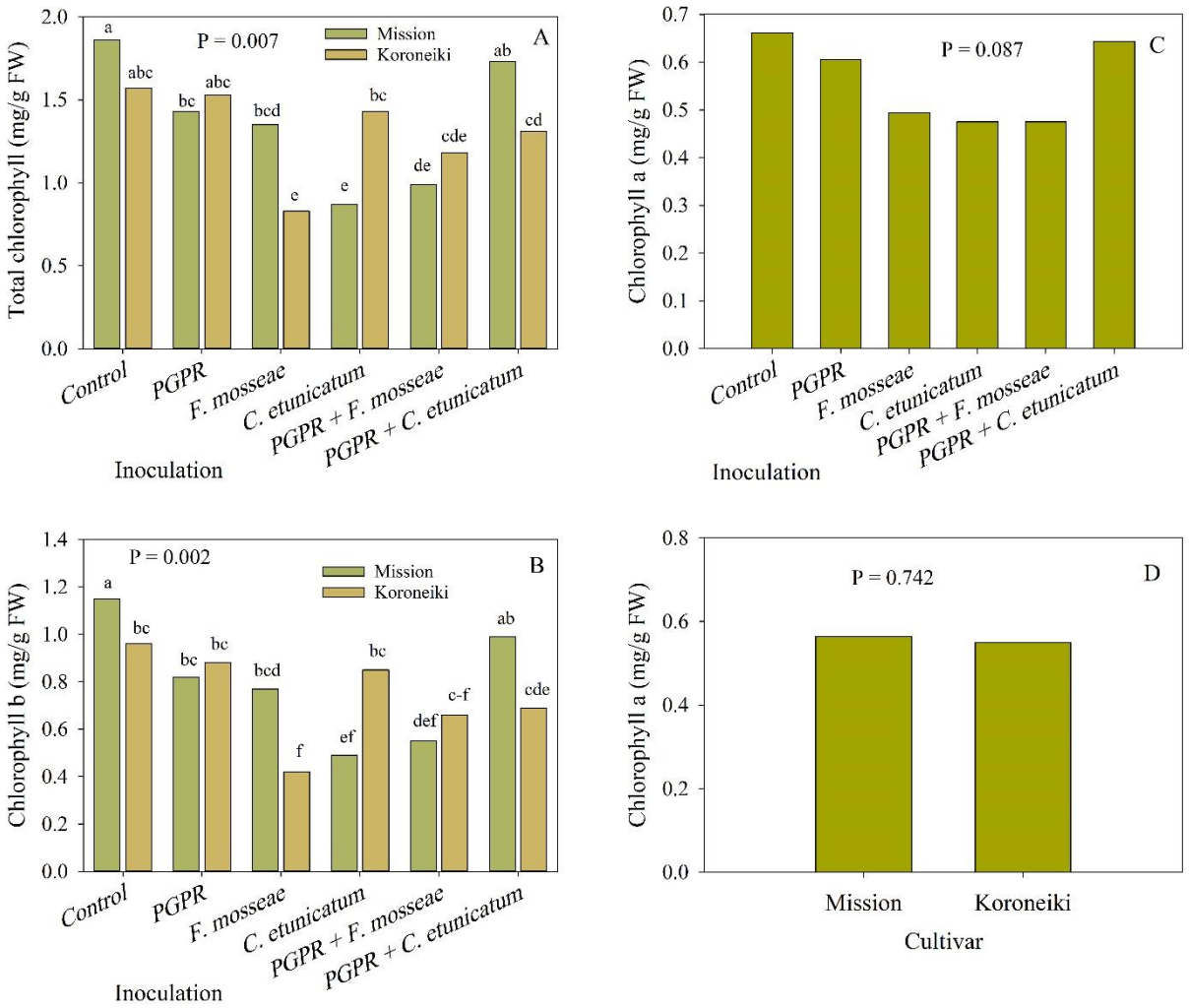

Fig. 4. The effects of inoculation treatments and cultivar on the content of total chlorophyll (A), chlorophyll b (B), and chlorophyll a (C and D) in olive plantlets. Different letters represent significant differences at P=0.01, Duncan's multiple range test. The abbreviation is as following: PGPR (plant growth-promoting rhizobacteria).

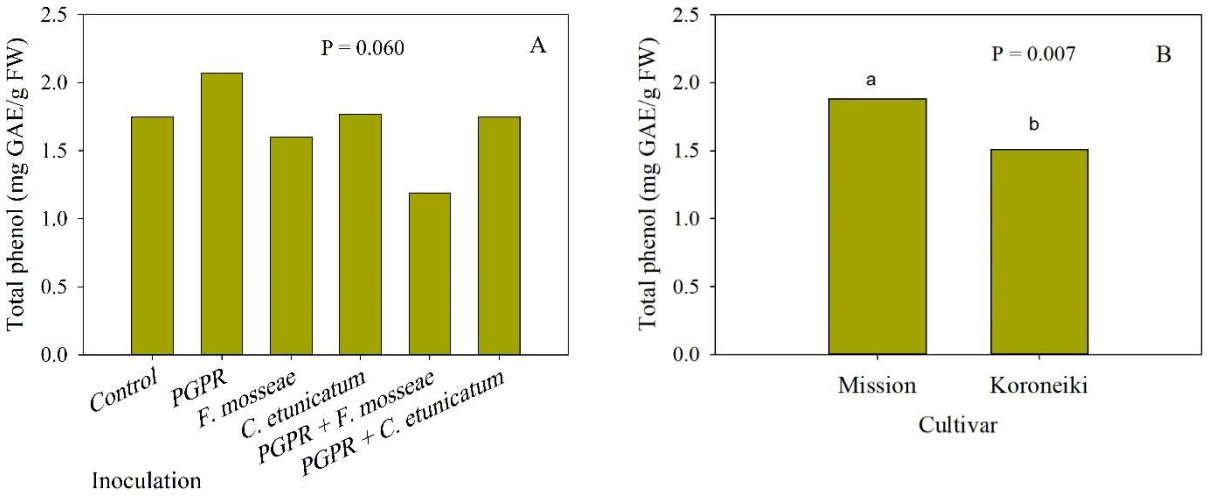

Fig. 5. The independent effects of inoculation treatments (A) and cultivar (B) on the total phenol content in olive plantlets. Different letters represent significant differences at P=0.01, Duncan's multiple range test. The abbreviation is as following: PGPR (plant growth-promoting rhizobacteria).

The analysis of variance showed that the interaction effect of cultivar and inoculation treatments on leaf total phenol was not significant. Additionally, no significant differences were observed in the amount of leaf total phenol in plants treated with AMF and PGPR (Fig. 5A). However, leaf total phenol content was higher in the 'Mission' cultivar (1.88 mg GAE/g FW) than in the 'Koroneiki' cultivar (1.51 mg GAE/g FW) (Fig. 5B). This suggests that the differences in leaf total phenol content were more influenced by the cultivar than by the inoculation treatments. Previous studies have reported conflicting results regarding the effects of AMF on leaf total phenol. Eftekhari et al. (2012) found that leaf total phenol in grapes increased after AMF inoculation compared to the control. In contrast, Ganz et al. (2002) reported no significant impact of AMF on leaf total phenol in olive. These inconsistencies suggest that the response of leaf total phenol to AMF may depend on factors such as plant species, cultivar, or environmental conditions (Hajiboland, 2013).

CONCLUSION

The combination of PGPR inoculation and IBA resulted in a significantly higher rooting rate and more roots per cutting. These findings suggest that PGPR can enhance root initiation and growth by modulating auxin signaling and metabolism, highlighting the synergistic potential of PGPR and auxin in promoting adventitious rooting. The second experiment demonstrated that the inoculation of *F. mosseae* had the most significant positive effect on the growth attributes of the 'Koroneiki' cultivar, while PGPR + *F. mosseae* led to enhanced growth in the 'Mission' cultivar. These findings emphasize the substantial benefits AMF and PGPR inoculation on the growth and development of olive plantlets, with cultivar-specific responses. The insights gained can inform the development of sustainable and tailored biofertilization approaches for olive production. The synergistic potential of PGPR and auxin in promoting adventitious rooting, as well as the cultivar-specific responses to combined inoculation of PGPR and AMF, provide valuable knowledge to optimize olive propagation and growth through the utilization of beneficial soil microorganisms. Further research is warranted to optimize the use of beneficial soil microorganisms for the successful propagation and establishment of olive plantlets.

Conflict of interest

The authors declare that there is no conflict of interest.

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