Greenhouse Plant Production Journal

Interactive Effects of Indole-3-butyric Acid, Arbuscular Mycorrhizal Fungi, and Rooting Media on Rooting of *Vitis vinifera* L. cv. Sistan Yaghooti Cuttings

Fatemeh Bidarnamani[a], Zeinab Mohkami[a], Ehsan Elahi-Moghaddam[b]*

[a]*Agriculture Institute, Research Institute of Zabol, Zabol, Iran*
[b]*Master of Natural Resources, Fisheries, Agriculture Institute, Research Institute of Zabol, Zabol, Iran*

Original Article

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Citation: Bidarnamani, F., Mohkami, Z. and Elahi-Moghaddam, E. 2026. Interactive Effects of Indole-3-butyric Acid, Arbuscular Mycorrhizal Fungi, and Rooting Media on Rooting of *Vitis vinifera* L. cv. Sistan Yaghooti Cuttings. Greenhouse Plant Production Journal 3(4): 20-31.

https://doi.org/10.22034/jgpp.2026.742909

KEYWORDS

Adventitious roots
Cocopeat
Glomus intraradices
Glomus mossaea
Vegetative Propagation
Yaghooti

ABSTRACT

Efficient vegetative propagation of grapevine depends on successful adventitious root formation, which is influenced by genotype, auxin supply, rooting environment, and biological interactions. The present study evaluated the individual and interactive effects of rooting medium, indole-3-butyric acid (IBA), and arbuscular mycorrhizal fungi (AMF) on the rooting performance of Vitis vinifera L. cv. Sistan Yaghooti cuttings. A three-factor factorial experiment was conducted under a completely randomized design using three rooting media (sand, soil, and cocopeat + perlite), three IBA concentrations (0, 2000, and 4000 mg L^{-1}), and four fungal treatments (non-inoculated control, Glomus intraradices, G. mossaea, and SuperMychoPlus). Rooting success and root-system traits were evaluated after approximately two months. Analysis of variance revealed significant effects and interactions among rooting medium, IBA concentration, and fungal treatment for several rooting traits, including significant three-way interactions. These results indicate that rooting responses strongly depended on the specific combination of IBA concentration, fungal treatment, and rooting medium. For example, the sand + 2000 mg L^{-1} IBA combination averaged 2.42 rooted cuttings per experimental unit, corresponding to 40.3% rooting, whereas the soil + untreated control averaged 0.58 rooted cuttings (9.7%). Several cocopeat + perlite combinations supported greater development of root-system traits, including root number and total root length. Thus, rooting success and root-system development did not necessarily follow the same response pattern. Overall, the findings indicate that propagation of Sistan Yaghooti grapevine should be evaluated using integrated treatment combinations rather than considering IBA concentration, fungal treatment, or rooting medium independently.

ARTICLE HISTORY

Received: 12 September 2026
Revised: 07 October 2026
Accepted: 20 October 2026

* Corresponding author: E. Elahi-Moghaddam
E-mail address: eemoghaddam62@gmail.com

Publisher: Arak University

1. Introduction

Grapevine (*Vitis vinifera* L.) is an economically important perennial fruit crop, and the production of healthy, uniform, and vigorous planting material is essential for successful vineyard establishment and sustainable grape production (Keller, 2020; Waite *et al*., 2015). The Yaghooti grape is the most important garden product in Sistan County, Sistan and Baluchestan Province, Iran, and plays a significant role in the region's economy and way of life (Fartout Enayat *et al*., 2023). Vegetative propagation by cane cuttings is widely used in grapevine production because it preserves the genetic characteristics of elite genotypes and provides a relatively simple and economical method for producing large numbers of plants, although rooting capacity can vary considerably among genotypes and propagation conditions (Waite *et al*., 2015; Arab *et al*., 2025). Recent propagation studies have further emphasized the importance of optimizing cutting type, misting, substrate, and environmental conditions to improve rooting and subsequent establishment of grapevine plants (Malai *et al*., 2024; Arab *et al*., 2025). Adventitious root formation in cuttings is a complex developmental process regulated by interactions among auxins, carbohydrate availability, nutrient status, and environmental conditions (Druege *et al*., 2019; Adem *et al*., 2024). Indole-3-butyric acid (IBA) is one of the most used synthetic auxins for stimulating adventitious rooting because of its effectiveness and relative stability, but its response depends on genotype, cutting characteristics, concentration, and rooting environment (Koukourikou-Petridou *et al*., 2018; Druege *et al*., 2019). In grapevine, IBA has been shown to influence rhizogenesis and root-system characteristics, with the magnitude of the response varying according to the genotype and position of the cutting on the cane (Koukourikou-Petridou *et al*., 2018). Recent studies have continued to confirm concentration-dependent responses to IBA in grapevine rootstocks and cultivars, although the most effective concentration differs among propagation systems (Hussein and Zaili, 2025; Moraes and Ono, 2026). Kumar Ausari *et al*. (2023) also reported improved growth of grape hardwood cuttings following IBA treatment in combination with different growing media. The physical properties of the rooting medium are equally important because water retention, drainage, aeration, temperature, and mechanical resistance directly influence the initiation and development of adventitious roots (Hartmann *et al*., 2011). Recent research has confirmed substantial differences among rooting media in grapevine, with perlite, pumice, and river sand producing markedly higher rooting percentages and root development than peat in ‘Dimrit’ grapevine (Uzun *et al*., 2025). Moreover, recent work on grapevine propagation demonstrated that perlite–cocopeat mixtures can provide a practical substrate for efficient production of grapevine plants under greenhouse conditions (Arab *et al*., 2025). These findings support the need to evaluate auxin treatments together with substrate conditions rather than assuming that a single IBA concentration will perform similarly across rooting environments. Bidarnamani *et al*. (2016) reported that rooting of *Schefflera* cuttings was promoted by perlite combined with *G. mosseae*, further illustrating the importance of interactions between biological inoculants and rooting media.

In addition to auxin and substrate conditions, arbuscular mycorrhizal fungi (AMF) may contribute to root development by improving nutrient acquisition, modifying root architecture, and influencing physiological and hormonal processes in host plants (Begum *et al*., 2019; Smith & Read, 2008). Recent evidence specifically from grapevine cuttings demonstrated that AMF inoculation can enhance root length, root surface area, root volume, nutrient acquisition, photosynthesis, and antioxidant activity, although the magnitude and direction of the response differed substantially among fungal species (Peng *et al*., 2025). In that study, *Funneliformis mosseae* strongly promoted root-system development and phosphorus acquisition, whereas *Paraglomus occultum* produced inhibitory effects on several growth and root traits, highlighting the importance of fungal identity and plant–fungus compatibility (Peng *et al*., 2025). A recent review also emphasized that grapevine–mycorrhiza interactions are strongly influenced by fungal identity, environmental conditions, and vineyard management, and that responses should not be generalized across inoculants or production systems (Valez *et al*., 2025). The potential interaction between auxin, AMF, and rooting medium is particularly important because these factors regulate different but interconnected components of adventitious root formation, including hormonal signaling, nutrient availability, water relations, and the physical environment surrounding developing roots (Druege *et al*., 2019; Hartmann *et al*., 2011; Adem *et al*., 2024). Nevertheless, most previous grapevine propagation studies have examined auxin treatments, rooting media, or AMF inoculation separately, and limited information is available on their combined effects in specific grape genotypes (Koukourikou-Petridou *et al*., 2018; Uzun *et al*., 2025; Peng *et al*., 2025). Therefore, factorial evaluation of these factors may provide a more realistic basis for identifying genotype-specific propagation protocols. To the best of our knowledge, no previous study has investigated the combined effects of arbuscular mycorrhizal fungi (AMF), auxin, and rooting substrate on the rooting of ‘Sistan Yaghooti’ grapevine cuttings. Given the potential beneficial effects of AMF on root development and the recurrent drought and water scarcity in the Sistan region, the present study was conducted to evaluate the effects of different combinations of these treatments on rooting performance and, subsequently, to assess the adaptation and establishment of rooted cuttings under water-stress conditions. This aspect is particularly important because a considerable proportion of rooted cuttings produced in other provinces, where climatic conditions differ substantially from those of Zabol, may exhibit poor survival and establishment after transplantation to the Sistan region. Therefore, developing a

propagation strategy that produces well-rooted and better-adapted cuttings may contribute to improving the establishment of 'Sistan Yaghooti' grapevines under the water-limited conditions of the region.

Material and Methods

2.1. Experimental site, plant material, and experimental design

The experiment was conducted during winter 2026 in the propagation greenhouse of the Agriculture Institute, Research Institute of Zabol, Iran. Uniform cuttings of *Vitis vinifera* L. cv. Sistan Yaghooti were prepared at approximately 20 cm in length and with 4–6 nodes per cutting in middle of February 2026. Cuttings were obtained during the dormant season; however, because of limitations in obtaining the plant material, the cuttings were received later than the intended collection period. The experiment consequently extended into a period of increasing temperatures in Zabol. The experiment was arranged as a three-factor factorial based on a completely randomized design (CRD). The first factor was rooting medium with three levels: sand, soil, and cocopeat + perlite. The second factor was IBA (Merk, made in Germany) concentration at 0, 2000, and 4000 ppm. The third factor was mycorrhizal fungi (from Zist Fanavar Toran Company, Shahrood, Iran) treatment with four levels: non-inoculated control, *Glomus intraradices*, *Glomus mosseae*, and Super-mycho plus. Each treatment combination was replicated three times, giving a total of 108 experimental units (3 × 3 × 4 × 3). Each experimental unit contained six cuttings, and the mean response of the six cuttings was used as a single experimental observation in the statistical analysis; therefore, the analysis was based on three independent replicates per treatment combination.

2.2. Cutting preparation, treatments, and propagation conditions

The required amount of indole-3-butyric acid (IBA) was first dissolved in a few drops of sodium hydroxide (NaOH), and the solution was then brought to the desired final volume with distilled water. The basal ends of the cuttings assigned to the IBA treatments were treated by a quick-dip method for 10 s using the respective IBA solutions, whereas untreated cuttings served as the 0 ppm control. Cuttings were planted in plastic seedling pots containing the assigned rooting medium and were maintained under greenhouse propagation conditions. AMF treatments were applied 20g per pot (It was mixed with the rooting medium in the upper half of the pot.). Because AMF colonization was not assessed microscopically, successful root colonization was not assumed in the interpretation of the results. The cuttings were maintained under an intermittent mist propagation system. Root-zone temperature was maintained at 24 ± 1 °C using bottom heating and the pots containing the cuttings were placed on the heating pipes, greenhouse air temperature was maintained at 20-25 °C (On most days) and 15°C in night, and relative humidity was maintained at 60–70% to reduce cutting desiccation during the rooting period. Due to the late timing of cutting collection in the hot Zabol region, air temperature inside the greenhouse occasionally reached 30–32 °C during some hours of the day.

2.3. Measurements and statistical analysis

After 2 months of propagation, the number of rooted cuttings was recorded for each experimental unit. Root-system traits were assessed as number of roots per rooted cutting, length of the largest root per cutting, and total root length per cutting. Data were analyzed using SAS software as a three-factor factorial experiment under a completely randomized design. The factorial analysis of variance tested the main effects of rooting medium, IBA concentration, and fungal treatment, together with all two-way and three-way interactions. Mean comparisons were performed using Duncan's multiple range test at the 5% probability level. Because significant interactions were detected for the evaluated traits, interpretation of treatment means was based primarily on the corresponding interaction effects rather than on main effects alone.

Result

The rooting results are presented in table 1.

3.1. Rooting percentage

The analysis of variance showed that rooting medium, IBA concentration, and fungal treatment significantly affected on rooting percentage, although the magnitude of the response depended strongly on treatment combinations (table 1).

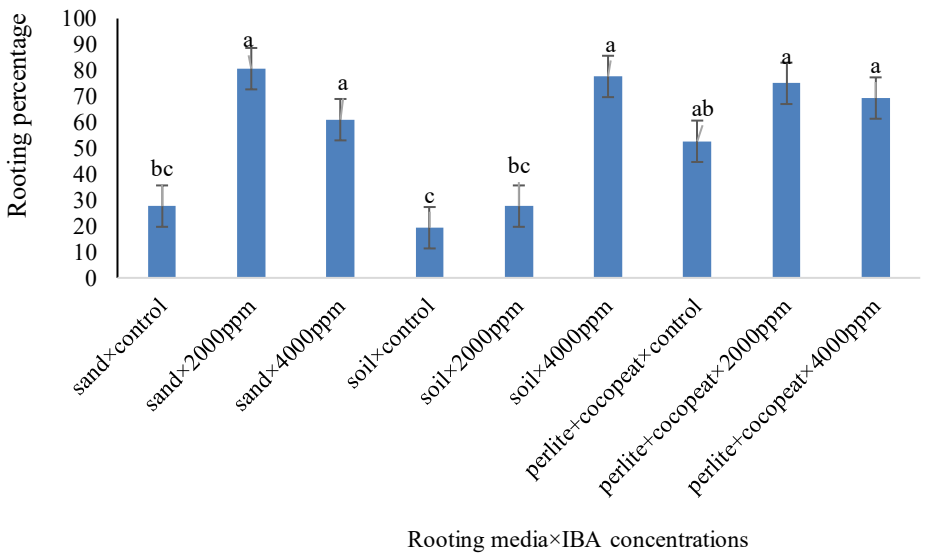


Figure 1. Interaction of rooting media and IBA hormone concentration on rooting percentage of grape cuttings

The highest rooting percentage was observed in the sand + 2000 mg L^{-1} IBA treatment (80.66%), which did not differ significantly from sand + 4000 mg L^{-1} IBA (77.67%). The control treatments of sand and soil without IBA had the lowest rooting percentages (19.33% and 27.67%, respectively) in Figure 1.

Table 1. Analysis of variance for rooting traits of Vitis vinifera L. cuttings

Treatments	Freedom degree	Rooting percentage	Number of roots	Length of largest root	Total length of roots
Rooting media	2	1327**	63.1*	7341**	124113**
IBA concentrations	2	1971**	91.5**	2338*	70555**
Mycorrhiza fungi	3	2708*	26.3^{ns}	1437^{ns}	31612^{ns}
media× IBA	4	723**	41.6*	2972**	49839*
media× fungi	6	1570**	43.9**	1565*	52953*
IBA × fungi	6	345**	20.6^{ns}	2034**	38804^{ns}
media× IBA × Fungi	12	468**	30.2*	2985**	56681**
Error	72	38.9	15.4	714	22147
Coefficient of Variation (CV)	-	19.18	13.63	13.72	17.02

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

According to Figure 2, the highest and lowest rooting percentages were observed in the cocopeat + perlite × F. mosseae treatment (81.33%) and soil × control treatment (29.67%), respectively. Among the non-inoculated controls, sand showed a higher rooting percentage than cocopeat + perlite and soil. The magnitude of the fungal response varied among rooting media, indicating that the effect of AMF was conditional on the propagation environment. The interaction between IBA and fungal treatments showed substantial variation among combinations (Figure 3). The untreated, non-inoculated control had the lowest rooting response, whereas several combinations involving IBA and SuperMychoPlus showed high rooting percentages. The soil + 4000 mg L^{-1} IBA + SuperMychoPlus combination reached 100% rooting in the observed treatment means. All two-way interactions (medium × IBA, medium × fungi, and IBA × fungi) and the three-way interaction were significant (Table 1). Therefore, the response to IBA varied according to the rooting medium and fungal treatment. The interaction graphs showed clear variation among treatment combinations, with several combinations assigned to different statistical groups according to Duncan's test. These results support interpretation at the interaction level rather than treating IBA, AMF, or rooting medium as universally independent effects.

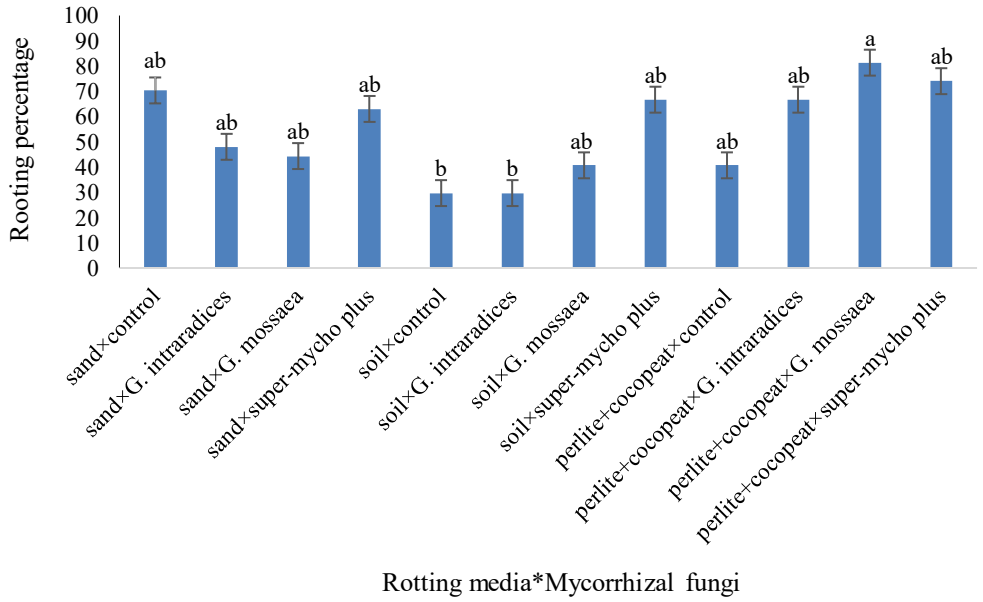


Figure 2. Interaction of rooting media and mycorrhizal fungi on rooting percentage of grape cuttings

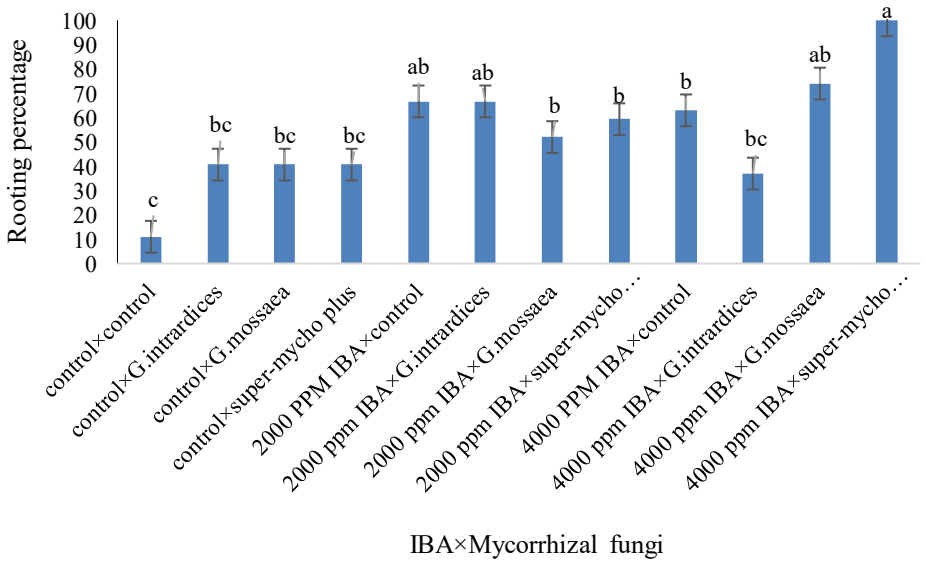


Figure 3. Interaction of IBA hormone concentration and mycorrhizal fungi treatments on rooting percentage of grape cuttings

3.2. Number of roots in each rooted cutting

Rooting medium and IBA concentration had significant main effects on the number of roots per rooted cutting, whereas the main effect of fungal treatment was not significant (Table 1). However, the medium × IBA, medium × fungi, and medium × IBA × fungi interactions were significant, demonstrating that fungal effects on root number were conditional on both substrate and auxin treatment. The interaction graphs indicate pronounced differences among treatment combinations, with the highest root number occurring in selected combinations involving the more favorable rooting environment and appropriate IBA level. In contrast, several combinations produced fewer roots and were assigned to lower statistical groups. These results indicate that increasing IBA concentration or applying AMF did not uniformly increase root number across all media.

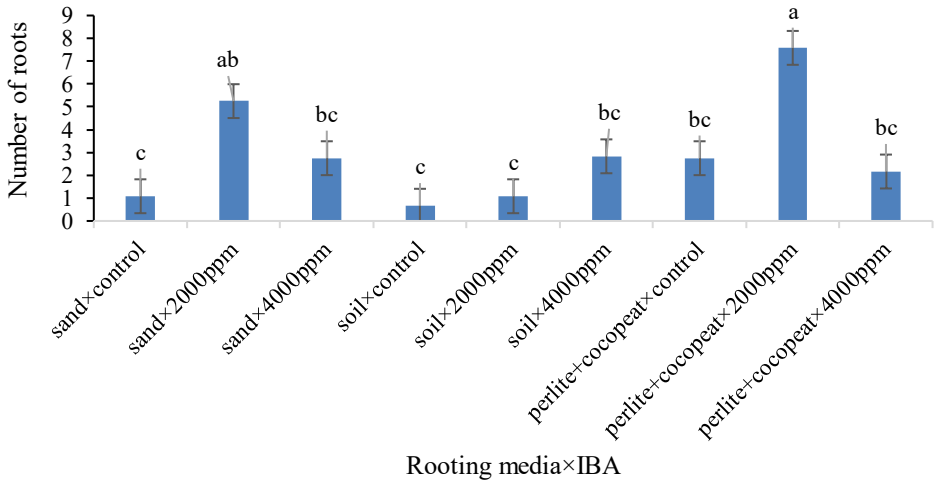


Figure 4. Interaction of rooting media and IBA on number of roots in each rooted cuttings of grape

The cocopeat + perlite × 2000 mg L−1 IBA combination had the highest numerical mean for number of roots among the medium × IBA combinations (7.58 roots per rooted cutting), and it was not significantly different from sand × 2000 mg L−1 IBA according to the displayed grouping. The soil × control combination had the lowest numerical mean (0.67 roots per rooted cutting). These comparisons are restricted to the two-way interaction because the three-way interaction was also significant (figure 4).

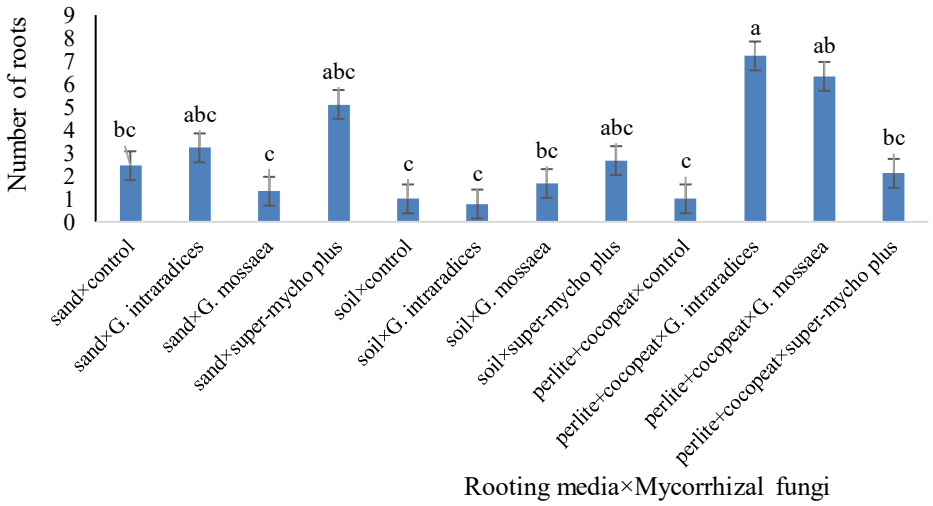


Figure 5 interaction of rooting media and mycorrhizal fungi on number of roots in each rooted cuttings of grape

Application of 2 species of mycorrhizal fungi in perlite+cocopeat showed the best result on number of roots in grape (figure 5). In the medium × fungal interaction, cocopeat + perlite combined with *G. intraradices* or *G. mosseae* showed relatively high numerical means for root number (7.22 and 6.33 roots per rooted cutting, respectively). The statistical grouping should be used to determine which of these differences are significant; the results should not be described as a general superiority of the two fungal species across all media.

3.3. Total length of roots

Total root length was significantly affected by rooting medium and IBA concentration, whereas the main effect of fungal treatment was not significant (Table 1). Nevertheless, significant medium × IBA, medium × fungi, and three-way interactions were detected, confirming that the development of the complete root system was strongly dependent on the specific combination of treatments. The interaction graphs show that some cocopeat + perlite combinations produced the greatest total root development, whereas several soil-based combinations produced comparatively shorter total root systems. The presence of significant interaction terms also indicates that the ranking of IBA and fungal treatments changed across rooting media, emphasizing the importance of evaluating total root length at the interaction level.

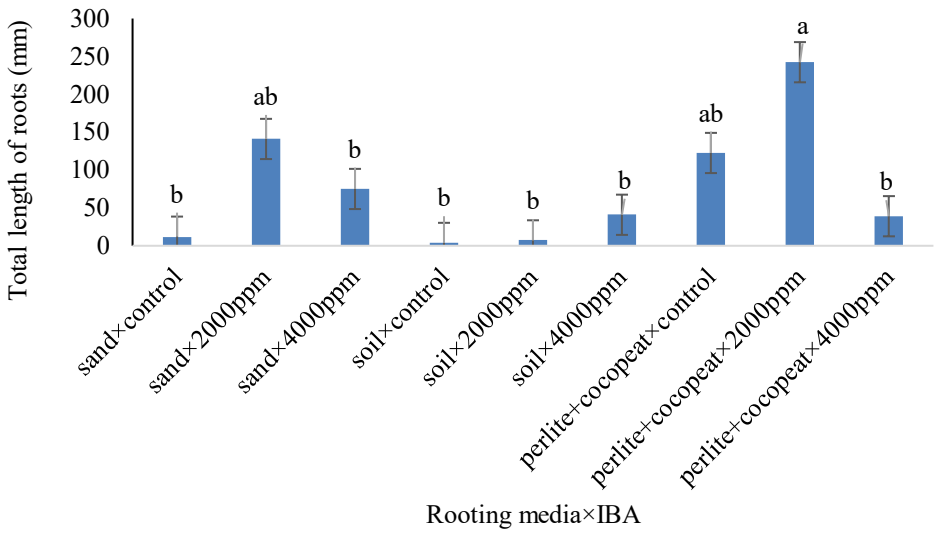


Figure 6. Interaction of rooting media and IBA on total length of rooted cuttings in grape

The cocopeat + perlite × 2000 mg L−1 IBA combination had the highest numerical mean for total root length (242.33 mm) among the medium × IBA combinations, whereas several soil-based combinations had much lower numerical means. Because the medium × IBA × fungal interaction was significant, these two-way comparisons describe patterns within the marginal interaction and should not be interpreted as a universal optimum (figure 6).

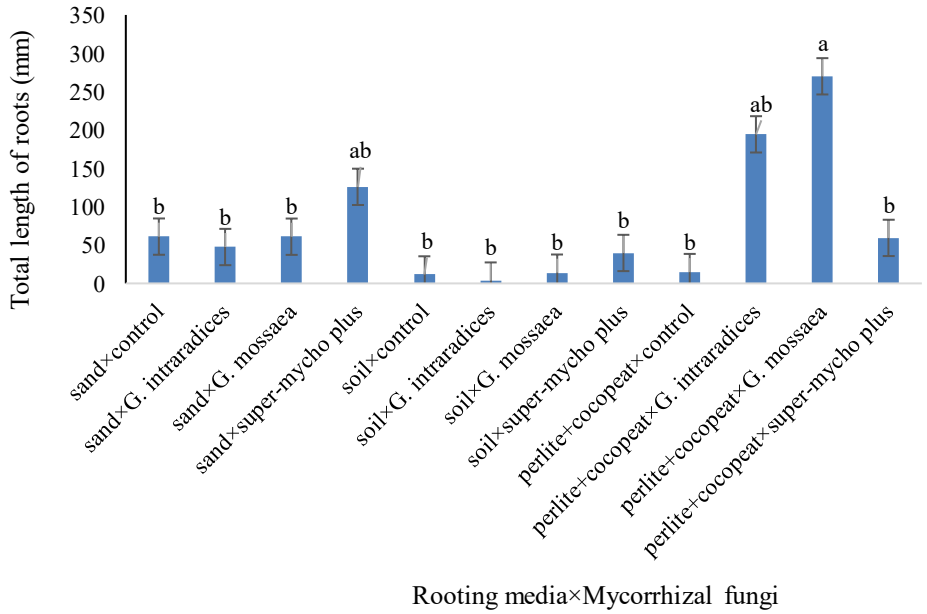


Figure 7. Interaction of rooting media and mycorrhizal fungi on total length of roots in grape

Within the medium × fungal interaction, cocopeat + perlite combined with *G. intraradices* and *G. mosseae* produced relatively high numerical means for total root length (194.33 and 270.00 mm, respectively). These values describe the marginal two-way interaction; they do not establish that either fungus was generally superior across all IBA concentrations (figure 7).

3.4. Length of largest root in each cutting

The length of the largest root was significantly influenced by rooting medium and IBA concentration, while the main effect of fungal treatment was not significant (Table 1). In contrast to root number and total root length, all relevant two-way interactions, including IBA × fungi, were significant, and the three-way interaction was also highly significant. The interaction graphs therefore demonstrate that elongation of the dominant root was highly treatment-specific. Some combinations produced markedly longer maximum roots, particularly under favorable

substrate conditions and appropriate IBA treatments, whereas other combinations were statistically inferior. The significant IBA × fungi interaction further suggests that the response of root elongation to IBA depended on the fungal inoculant applied.

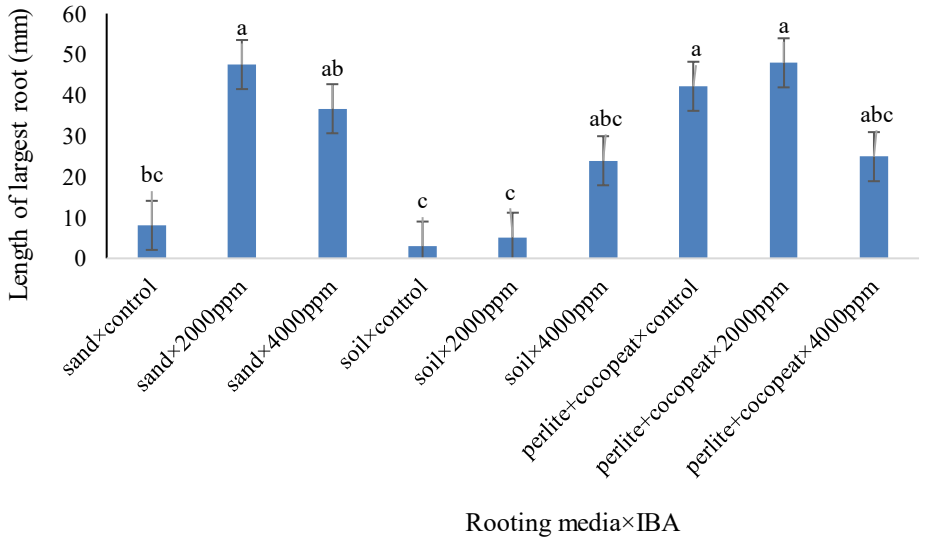


Figure 8. Interaction of rooting media and IBA on length of largest root in grape cuttings

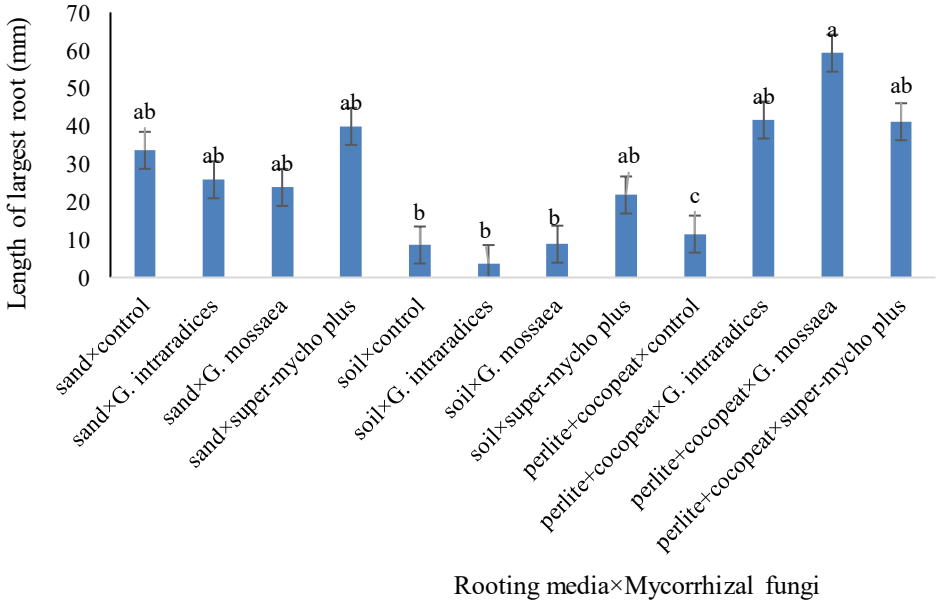


Figure 9. Interaction of rooting media and mycorrhizal fungi on length of largest roots in grape

Within the medium × IBA interaction, the numerical means for largest-root length were generally greater in sand and cocopeat + perlite than in soil. For example, cocopeat + perlite × 2000 mg L−1 IBA had a mean largest-root length of 47.92 mm, compared with 24.92 mm at 4000 mg L−1 IBA in the same medium. These values indicate a concentration-dependent response within this interaction, rather than evidence that 2000 mg L−1 IBA is universally optimal (figure 8). Within the medium × fungal interaction, cocopeat + perlite × *G. mosseae* had the highest numerical mean for largest-root length (59.22 mm), followed by cocopeat + perlite × *G. intraradices* (41.56 mm) and Super-mycho plus (41.11 mm). The corresponding statistical groupings should be used when determining which pairwise differences are significant (figure 9).

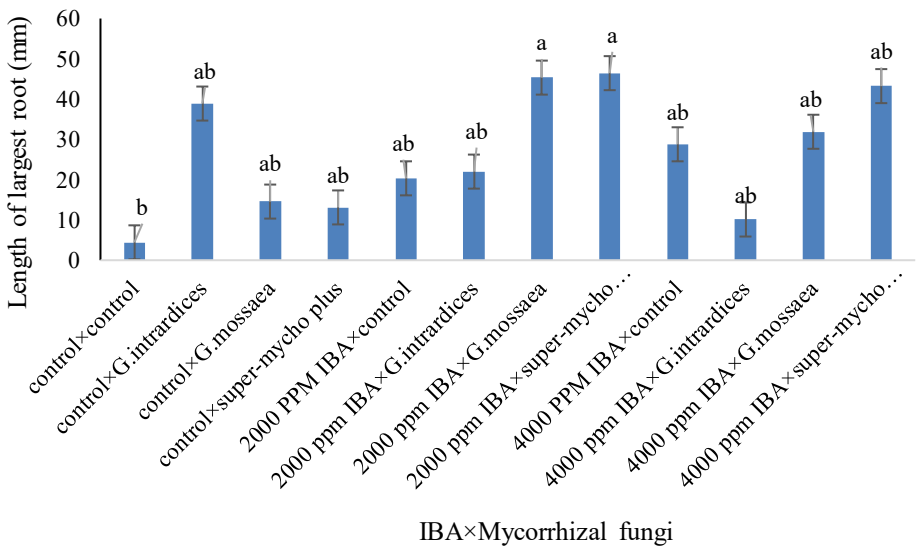


Figure 10. Interaction of IBA and mycorrhizal fungi on length of largest root in grape cuttings

The IBA × fungal interaction also showed treatment-specific differences in largest-root length. The highest numerical means were observed for 2000 mg L−1 IBA combined with *G. mosseae* or Super-mycho plus, while the 0 mg L−1 IBA control combined with no fungal inoculation had the lowest numerical mean. Because the three-way interaction was significant, these marginal means should be interpreted as conditional patterns rather than as evidence for a universally best IBA–fungus combination. Cuttings without IBA and mycorrhizal fungi showed the lowest length of roots (figure 10). Differentiation in rooting show in figure 11.



Figure 11. Rooting of cuttings in some treatments

The best rooting percentage showed in soil+4000 ppm IBA+super-mycho plus that it had not significant differences with sand+2000 ppm IBA+ *G. intraradices* (table 2). Also, the greatest number of roots was in soil+4000 ppm IBA+Super-mycho plus (4.67 roots). Length of largest root and total length of roots in each rooted cutting showed perlite+cocopeat with 2000 ppm IBA and G. intraradices was better than other treatments. In other words, the lighter growing medium resulted in better root development in this experiment. On the other hand, the treatments without IBA and Mycorrhizal fungi in sand and soil media had the shortest roots between others.

Discussion

The present study demonstrated that rooting responses of *Vitis vinifera* L. cv. Yaghooti depended strongly on the specific combination of rooting medium, IBA concentration, and fungal treatment. The significant two-way and three-way interactions observed for several rooting traits indicate that the effect of one factor could not be interpreted independently of the others. Therefore, the results should be considered primarily in terms of treatment combinations rather than as evidence for a universally superior IBA concentration, fungal treatment, or rooting medium. This interaction-dependent response is consistent with the complex regulation of adventitious root formation, in which auxin status interacts with environmental and biological factors during root initiation and development (Druege *et al*., 2019; Adem *et al*., 2024).

Table 2. Mean comparison of significant traits in 3 factors

Treatments			Traits			
Media	IBA (ppm)	Mycorrhizal fungi	Rooting percentage	Number of roots	Length of largest root (mm)	Total length of roots (mm)
Sand	0	control	5.56^{f}	0.3333^{f}	1.67^{f}	1.7^{d}
		G. mossaea	11.11^{def}	1.3333^{cdef}	16^{def}	16^{d}
		G. intraradices	16.67^{def}	0.6667^{ef}	5.67^{ef}	9^{d}
		Super-mycho plus	16.67^{def}	1^{def}	9^{def}	21.3^{d}
	2000	control	5.56^{f}	4^{ab}	51	91.67^{cd}
		G. mossaea	50^{bc}	2^{cdef}	41.67	95^{cd}
		G. intraradices	66.67^{ab}	0.6667^{ef}	5.67^{ef}	7.5^{d}
		Super-mycho plus	44.44^{bc}	3^{bc}	91.67^{abc}	325.7^{bc}
	4000	control	11.11^{def}	2^{cdef}	48^{cdef}	89.7^{cd}
		G. mossaea	27.78^{cdef}	1^{def}	19.67^{def}	31.3^{d}
		G. intraradices	33.33^{cdef}	2.6667^{bcd}	60^{bcd}	148.3^{bcd}
		Super-mycho plus	27.78^{cdef}	1.6667^{cdef}	19^{def}	30.7^{d}
Soil	0	control	5.56^{ef}	0.3333^{f}	1.67^{f}	1.7^{d}
		G. mossaea	11.11^{def}	0.3333^{f}	1.67^{f}	1.7^{d}
		G. intraradices	11.11^{def}	1^{def}	6.67^{def}	9.7^{d}
		Super-mycho plus	22.22^{ef}	0.6667^{ef}	2^{f}	2^{d}
	2000	control	5.56^{ef}	0.6667^{ef}	4.33^{ef}	4.3^{d}
		G. mossaea	11.11^{def}	1^{def}	2.67^{f}	2.7^{d}
		G. intraradices	11.11^{def}	1^{def}	8.67^{def}	14.3^{d}
		Super-mycho plus	27.78^{cdef}	0.6667^{ef}	5^{ef}	7^{d}
	4000	control	16.67^{ef}	6667^{cdef}	19.67^{def}	29^{d}
		G. mossaea	27.78^{cdef}	1.3333^{cdef}	6.67^{def}	6.7^{d}
		G. intraradices	16.67^{def}	1.6667^{cdef}	11^{def}	18^{d}
		Super-mycho plus	77.78^{a}	4.6667^{a}	58.67^{bcde}	110^{cd}
Perlite+ Cocopeat	0	control	5.56^{ef}	0.3333^{f}	10^{def}	13.3^{d}
		G. mossaea	50^{bc}	2^{cdef}	99^{ab}	391.3^{b}
		G. intraradices	33.33^{cdef}	2^{cdef}	31.33^{def}	47.3^{cd}
		Super-mycho plus	16.67^{ef}	2^{cdef}	28.33^{def}	38^{cd}
	2000	control	33.33^{cdef}	1.3333^{cdef}	5.67^{ef}	5.7^{d}
		G. mossaea	27.78^{cdef}	3^{bc}	21.67^{def}	186.7^{bcd}
		G. intraradices	22.22^{ef}	3^{bc}	121.67^{a}	723.3^{a}
		Super-mycho plus	38.89^{cde}	1.6667^{cdef}	42.67^{cdef}	53.7^{cd}
	4000	control	33.33^{cdef}	2^{cdef}	18.67^{def}	25.3^{d}
		G. mossaea	33.33^{cdef}	1^{def}	4^{f}	5^{d}
		G. intraradices	50^{bc}	2.3333^{cde}	24.67^{def}	39.3^{cd}
		Super-mycho plus	50^{bc}	3^{bc}	52.33^{bcdef}	86.3^{cd}

The treatments with same significant letter had not significant differences together.

Rooting percentage varied substantially among the rooting-medium × IBA combinations. The highest rooting percentage was obtained with sand + 2000 ppm IBA (80.66%), followed by sand + 4000 ppm IBA and soil + 4000 ppm IBA (both 77.67%). In contrast, the lowest values occurred in the untreated combinations, particularly soil + 0 ppm IBA (19.33%) and sand + 0 ppm IBA (27.67%). These results indicate that IBA improved rooting under some substrate conditions, although the magnitude of the response depended on the rooting medium. Importantly, rooting percentage represents the proportion of cuttings that initiated roots and differs from root number or root length, which describe root-system development among successfully rooted cuttings (Waite *et al*., 2015; Arab *et al*., 2025). The effect of fungal treatment on rooting percentage also depended on the rooting medium. The highest mean in the rooting-medium × fungal interaction was recorded for cocopeat + perlite combined with *G. mossaea* (81.33%), whereas the lowest value occurred with soil + the non-inoculated control (29.67%). This variation indicates that fungal treatment responses were conditional on the physical and biological environment provided by the rooting substrate. Similar interaction-dependent responses have been reported for beneficial microorganisms and rooting media, suggesting that inoculant performance may vary among propagation environments. However, AMF colonization was not quantified in the present study; therefore, these differences should be interpreted as treatment-associated responses rather than direct evidence of successful mycorrhizal establishment (Begum *et al*., 2019; Peng *et al*., 2025).

The IBA × fungal interaction further demonstrated that some combinations produced high rooting percentages. The combination of 4000 ppm IBA + SuperMychoPlus reached 100% rooting in the marginal IBA × fungal interaction. However, this value represents the mean across the three rooting media and should not be interpreted as the rooting percentage of a specific three-factor treatment. When all three factors were considered simultaneously, the highest rooting percentage was 77.78% for soil + 4000 mg L^{-1} IBA + SuperMychoPlus. This distinction is important because the significant three-way interaction indicates that the response to the IBA–fungus combination varied according to the rooting medium. The concentration-dependent response to IBA is consistent with the recognized role of auxin in adventitious root induction and development. Although IBA is widely used in vegetative propagation, its effectiveness depends on genotype, cutting characteristics, physiological condition, concentration, application method, and environmental conditions. Previous studies in grapevine have likewise reported variation in the most effective IBA concentration among cultivars and propagation systems (Koukourikou-Petridou *et al*., 2018; Kumar Ausari *et al*., 2023; Hussein and Zaili, 2025). Thus, the relatively strong responses obtained with 2000 and 4000 ppm IBA in the present study should be considered specific to Sistan Yaghooti cuttings and the propagation conditions used rather than generally optimal concentrations for grapevine propagation.

Rooting medium also influenced subsequent root-system development. Although sand + 2000 mg L^{-1} IBA produced the highest rooting percentage in the medium × IBA interaction (80.66%), cocopeat + perlite combined with 2000 mg L^{-1} IBA produced the highest numerical means for root number (7.58 roots per rooted cutting) and total root length (242.33 mm) among the corresponding two-way interactions. Several cocopeat + perlite treatments also produced relatively high maximum root length. Thus, a substrate that promotes a high proportion of rooted cuttings does not necessarily produce the most developed root system. The physical properties of cocopeat–perlite mixtures, particularly their potential balance between water retention and aeration, may have favored root elongation and branching; however, these properties were not measured and therefore remain possible explanations rather than demonstrated mechanisms. Similar substrate-dependent responses have been reported in grapevine propagation studies (Arab *et al*., 2025; Uzun *et al*., 2025). The distinction between rooting percentage and root-system traits is important for evaluating the practical value of the treatments. Rooting percentage reflects the success of root initiation, whereas root number, total root length, and maximum root length describe root-system development after rooting has occurred. In the present study, sand + 2000 ppm IBA was particularly effective for rooting percentage, whereas several cocopeat + perlite combinations promoted greater development of root-system traits. Therefore, propagation treatments should not be selected solely on the basis of rooting percentage. A treatment that produces a high proportion of rooted cuttings may not necessarily produce the most vigorous root systems, and vice versa.

The effects of fungal treatments were more evident through their interactions than through a consistent main effect. Although fungal treatment significantly affected rooting percentage, its main effect was not significant for root number, maximum root length, or total root length. Significant interactions involving fungal treatment were nevertheless detected for several traits, indicating that its effects depended on the rooting medium and/or IBA concentration rather than representing a uniform stimulatory response. Differences among fungal species may be related to inoculum characteristics, host compatibility, substrate conditions, and the timing and extent of root colonization. Peng *et al*. (2025) similarly demonstrated differences among AMF species in their effects on rooting and root-system characteristics of grapevine cuttings. However, because AMF colonization was not assessed in the present study, the observed responses cannot be attributed definitively to successful root colonization or to a specific AMF-mediated mechanism. The interactions between IBA and fungal treatments for root number and root length may indicate that these treatments influenced different components of root development. Auxin plays a central role in adventitious root induction and initiation, whereas AMF may potentially affect nutrient acquisition, carbon allocation, hormonal signaling, and root development. Nevertheless, endogenous hormones, nutrient status, physiological parameters, and AMF colonization were not measured in this experiment. Therefore, the observed statistical interactions demonstrate differential treatment responses but do not establish the physiological mechanisms responsible for them. These mechanisms should be considered hypotheses requiring confirmation in future experiments (Druege *et al*., 2019; Adem *et al*., 2024; Peng *et al*., 2025).

Conclusion

The present study demonstrated that vegetative propagation of *Vitis vinifera* L. cv. Sistan Yaghooti is strongly influenced by the combined effects of rooting medium, IBA concentration, and mycorrhizal treatment. Rooting medium and IBA significantly affected all evaluated root traits, while fungal treatments contributed mainly through interaction effects, demonstrating that AMF responses were dependent on the auxin level and rooting environment. Significant two-way and three-way interactions were detected for the measured traits, confirming that no single factor can be considered independently when optimizing propagation. The highest individual rooting response was obtained with Super-mycho plus combined with 4000 ppm IBA in soil, whereas several combinations involving cocopeat + perlite generally supported superior root-system development. Therefore, the most effective propagation strategy for Sistan Yaghooti should be selected on the basis of integrated treatment combinations that

maximize both rooting success and root quality. Future experiments should verify these findings by measuring AMF colonization, substrate physicochemical properties, nutrient status, and post-transplant performance.

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