

Synergistic Effects of Mycorrhizae and Growth-Promoting Substances on the Success of Grafting and the Quality of Liberica Coffee Seedlings

Elis Kartika^{1,2} , Zulkarnain Zulkarnain^{1,3*} , Made Deviani Duaja¹ , Gusniwati Gusniwati¹ 

¹Department of Agroecotechnology, Faculty of Agriculture, University of Jambi, Kampus Pinang Masak, Jambi 36361, Indonesia

²Center of Excellence for Eco-Engineering and Education for Coffee, Local Innovation, and Multidisciplinary (E2KOLIM), Jambi 36361, Indonesia

³Center of Excellence for Biodiversity and Land-use Transformation Systems (BLaTS), Jambi 36361, Indonesia

*Corresponding author: dr.zulkarnain@yahoo.com

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ABSTRACT

Grafting is a vegetative propagation method that is commonly used to produce Liberica coffee seedlings and is popular among coffee growers in Jambi Province, Indonesia. However, coffee grafting is often hampered by the incompatibility of the scion and rootstock or the slow growth of grafted seedlings. This study aimed to evaluate the effects of mycorrhizal inoculation, along with the application of plant growth-promoting substances on the success of grafting and the quality of the seedlings. Six growth-promoting substances (IBA, BAP, coconut water, bean sprout extract, shallot extract, and a control) were applied to grafted seedlings grown in peat soil with or without mycorrhiza. The trial was arranged in a randomized block design with three replications, and ten seedlings were grown in each experimental unit. The highest success rate (93.33%) was achieved when the seedlings were treated with BAP and inoculated with mycorrhizae. The rate of mycorrhizal colonization of coffee roots ranged from 3.33 to 83.33%, with the highest rate associated with the application of BAP (83.33%) along with mycorrhizal inoculation and the lowest (3.33%) associated with the application of IBA, coconut water, and shallot extract without mycorrhizal inoculation. Our findings suggest that the application of plant growth-promoting substances and mycorrhizal inoculation are critical for improving the graft union and quality of Liberica coffee seedlings grown in peat soil. The application of natural growth-promoting substances such as coconut water, bean sprout extract, and shallot extract also contributed positively to seedling growth. However, their effects were weaker than those of synthetic substances.

Key words: *Coffea liberica*; plant propagation; plant hormones; arbuscular mycorrhizal fungi.

1 INTRODUCTION

Liberica coffee (*Coffea liberica*) is a high-potential coffee species widely grown by farmers in Tanjung Jabung Barat Regency, Jambi Province, Indonesia. Its distinctive flavor makes it sought after in both domestic and international markets, contributing to increased farmer incomes, the local economy, and the value of agricultural exports. High-quality seedlings are crucial for productive and sustainable coffee cultivation, influencing growth, stress resistance, and yields. One standard method for producing superior seedlings is vegetative propagation through grafting, which allows for the inheritance of superior traits from the parent plant, accelerates fruiting time, and increases productivity and resistance to biotic and abiotic stresses.

Grafting is a plant propagation method that is the primary method for propagating coffee seedlings (Carvalho et al., 2015; Espindula et al., 2018; Ferreira et al., 2009, 2011). However, the success rate of grafting depends heavily on several factors, including the physiological condition of the rootstock, the suitability of the scion, presence of growth-promoting substances, and the adaptation of the seedlings to the growth medium. In practice, many growth media for nurseries, such as peat soils, are naturally nutrient poor with a very low pH. This may prevent the optimal growth of rootstocks, ultimately affecting the success of the grafting process and the quality of the resulting seedlings.

One strategy for overcoming problems with growth media is to create symbiotic associations between soil fungi, known as mycorrhizae, and plant roots to expand the absorption area of roots, thereby improving the efficiency of water and nutrient uptake, especially that of phosphorus (Ashwin et al., 2018; Kartika et al., 2016, 2018, 2021, 2022; Kartika & Gusniwati, 2019; Suparno et al., 2015). In addition, mycorrhizae play a role in increasing plant resistance to various abiotic (Evelin et al., 2019; Frew & Price, 2019; Haque & Matsubara, 2018; Li et al., 2025; Liu et al., 2021; Santander et al., 2017), biotic (Ahmed et al., 2020; Campo et al., 2020; Deja-Sikora et al., 2020; Dowarah et al., 2021), and heavy metal (Atakan et al., 2018; Emamverdian et al., 2015; Gong & Tian, 2019) stresses and can increase plant competitiveness against weeds (El Omari & El Ghachtouli, 2021; França et al., 2016; Li et al., 2016). Mycorrhizae also produce growth hormones such as auxin, cytokinin, and gibberellin, which play important roles in plant physiological processes, including supporting callus formation and the successful union of rootstock and scion during grafting (Ahmed et al., 2024).

In addition to the mycorrhizae association, the success of grafting is strongly influenced by the presence of plant growth-promoting substances. Growth-promoting substances are active compounds that promote cell division and elongation, tissue differentiation and root and shoot formation. Auxin and

cytokinin, for example, play essential roles in the process of graft union. The appropriate application of growth-promoting substances can increase the success rate of grafting while accelerating callus growth between the rootstock and scion (Köse & Güleriyüz, 2006).

The interaction between mycorrhizae and growth-promoting substances can potentially improve both grafting success and seedling quality. Mycorrhizae not only enhance the efficiency of nutrient and water uptake but also increase plant sensitivity to growth-promoting substances or even modulate plant requirements for specific hormones (Alkaç et al., 2023; Ashour et al., 2021; Nahak et al., 2018). Conversely, the presence of growth-promoting substances can also influence mycorrhizal colonization and the quality of the symbionts formed.

Although numerous studies on the role of mycorrhizae and growth-promoting substances in plant growth have been reported separately, their combined effects on the grafting of *Liberica* coffee need further investigation. Therefore, this study aimed to evaluate the synergistic effects of mycorrhizae and plant growth-promoting substances on grafting success and the quality of *Liberica* coffee seedlings. The results of this study are expected to provide valuable scientific contributions and practical solutions for the development of *Liberica* coffee nurseries in peat soil. The developed technology could be more efficient, more sustainable, and better adapted to the challenges posed by suboptimal land conditions.

2 MATERIAL AND METHODS

2.1 Plant Materials

This study was conducted in Mekar Jaya Village, Betara District, Tanjung Jabung Barat Regency, Jambi Province, Indonesia. The rootstocks were 3-month-old *Liberica* coffee seedlings, 10–12 cm tall, with approximately 4 fully opened green leaves. The routine maintenance of rootstocks, such as weeding, watering, and pest and disease control, was carried out to ensure healthy growth. Inorganic fertilizers were applied at 50% of the recommended dosage: 2.31 g of urea (46% N), 1.43 g of SP-36 (36% P_2O_5), and 1.10 g of KCl (60% K_2O) per rootstock.

The cultivar used for both rootstock and scion was *Coffea liberica* cv ‘Kopi Liberika Tungkal Komposit’. The scions were collected from a scion garden in West Tanjung Jabung Regency, Jambi, Indonesia. The scions were collected in the morning, starting around 7:00 a.m., and then wrapped in wet paper and placed in airtight plastic bags before use. The grafted scions met the following criteria: originated from a 25–30 year-old mother plant, had one pair of leaves (each cut in half), had a stem diameter of 1–2 cm, and were approximately 15 cm long.

2.2 Media Preparation

The growth medium was peat soil. The soil was air-dried and sieved through a 10-mesh sieve. Afterward, it was sterilized to kill all organisms present in the soil, ensuring that only the inoculated mycorrhiza grew and developed, and that the observed response was due to the influence of only the mycorrhiza. The soil was sterilized in a simple way by steaming it in a traditional drum steamer containing boiling water heated by firewood. The fire was maintained so that the water remained at a boil during steaming for 10 hours.

2.3 Grafting and Maintenance

The rootstock was cut approximately 10 cm from the root collar and a split approximately 2 cm long was made in the top. The prepared scion was cut on both sides to make it pointed, and some of the leaves were removed. Afterward, the scion was inserted into the split made in the rootstock and it was closed with plastic rope. The grafted seedlings were kept under a plastic cover to protect them from heavy rain and high temperatures. Three weeks after grafting, the cover was removed, and the seedlings were maintained in a glasshouse. A routine plant care (watering, applying fertilizer, and controlling pests and diseases) was carried out carefully.

2.4 Mycorrhizal Inoculation

The inoculum used was an isolate of arbuscular mycorrhizae fungi (AMF) isolated from the rhizosphere of *Liberica* coffee plants grown in West Tanjung Jabung Regency. These isolates were collected from a peatland (Kartika et al., 2019). The isolate used was a combination of *Glomus* sp-1a and *Glomus* sp-3c. Mycorrhizal isolates were applied when the seedlings were transferred from small to larger polybags by pouring 10 g of inoculum (approximately 50 spores) around the root area of the seedlings.

2.5 Preparation of Growth-Promoting Substances

Growth-promoting substances such as IBA, BAP, coconut water, bean sprout extract, and shallot extract, were prepared in advance and applied before to the grafting process. Each IBA and BAP were tested at a concentration of 100 ppm prepared by dissolving 10 milligrams of each IBA and BAP separately in a few drops of alcohol, then adding distilled water to a volume of 100 mL. Bean sprout extract and shallot extract were prepared by grinding 0.5 kg of bean sprouts and shallot bulbs, respectively, then filtering and separating the extract from the pulp. Meanwhile, coconut water was obtained from young coconut. The volume of each growth-promoting substance used to soak the scion was 100 mL.

The scions were soaked in the growth-promoting substance solution for approximately 15 minutes and then dried using tissue paper. Once sufficiently dry, the grafting process was carried out.

2.6 Experimental Design

A factorial randomized block design was used in this trial. The factors tested were mycorrhizal inoculation (with and without mycorrhizae) and the application of growth-promoting substances (no growth-promoting substance or control, 100 ppm IBA, 100 ppm BAP, 25% coconut water, 25% bean sprout extract, and 25% shallot extract), and 12 treatment combinations. Each combination was replicated three times, resulting in 36 experimental units. Each experimental unit consisted of 10 grafts, four of which were randomly selected as samples.

2.7 Observation and Data Analysis

Observations were made on the following variables: root infection, grafting success rate, time of apical bud emergence, apical shoot diameter, total leaf area, P absorption and the seedling quality index. The observation on root infection was carried out staining method developed by Kormanik and McGraw (1982). Mycorrhizal colonization was observed under a microscope by observing all fields of view, marking with a (+) for colonization and a (-) for non-colonization. Ten fields of view were observed in one root section. The formula used to calculate the percentage of root colonization by mycorrhiza was as follows:

$$\text{Root infection (\%)} = \frac{\Sigma \text{field of views (+)}}{\Sigma \text{total field of views}} \times 100\%$$

The grafting success rate was calculated based on the percentage of grafted plants that survived until the end of the study, using the following formula:

$$\text{Grafting success rate (\%)} = \frac{\Sigma \text{survive graftings}}{\Sigma \text{total graftings}} \times 100\%$$

The time of bud emergence was determined by counting the number of days from the time of grafting until the apical bud appeared. The apical shoot diameter was measured at a height of 0.5-1.0 cm from the base of the shoot using a caliper. The total leaf area per plant was measured in length and width, and the leaf area was calculated using the formula (Gardner et al., 2017): Total Leaf Area = Length x Width x 0.67, where 0.67 is a constant value for perennial crops such as coffee and cocoa.

Phosphorus absorption was determined using the wet digestion method and the formation of a blue molybdate complex according to Jones (2001). Samples consisting of leaves, twigs, and stems were dried at 70°C to a constant weight, then finely ground. A total of 0.2 - 0.5 g of sample was digested using a mixture of concentrated acids HNO₃-HClO₄ (5:1, v/v) to obtain a clear solution, then diluted with distilled water to a volume of 50 mL. Part of the solution was reacted with ammonium molybdate and a reducing agent to form a blue complex, the absorbance of which was then measured using a spectrophotometer at a wavelength of 880 nm. Phosphorus content was determined based on a standard curve and expressed as a percentage of dry weight. To calculate the P absorbance, the formula was used:

$$\text{P absorption (mg)} = \text{P content (\%)} \times \text{Shoot dry weight}$$

Seedling quality index was determined according to Dickson et al. (1960) method based on the following formula:

$$\text{Seedling Quality Index} = \frac{\text{Total seedling dry matter}}{\left(\frac{\text{Stem height}}{\text{Stem diameter}} \right) + \left(\frac{\text{Shoot dry matter}}{\text{Root dry matter}} \right)}$$

To determine the effect of treatment on the observed variables, the data were statistically analyzed using analysis of variance (ANOVA) by the aid of DSASTAT Ver.1.515 application {Onofri, 2014 #67}, followed by the least significant difference (LSD) test at the 5% confidence level.

3 RESULTS

3.1 Root Infection

There was no interaction effect between the mycorrhizal and plant growth-promoting substance treatments on root infection (Table 1). The root infection rate of seedlings inoculated with mycorrhizae was 77.5%. Seedlings without mycorrhizae also had an infection rate of 5%, even though the growth medium had been sterilized.

3.2 Grafting Success Rate

There was a significant interaction between mycorrhizae and plant growth-promoting substances on grafting success rates. Compared with the application without mycorrhizal inoculation, the application of growth-promoting substances

Table 1: Mycorrhizal colonization (%) of Liberica coffee seedling roots following mycorrhizal inoculation and the application of plant growth-promoting substances.

Mycorrhizal inoculation	Control	IBA	BAP	Coconut water	Bean sprout extract	Shallot extract
Without mycorrhiza	6.67±2.89	3.33±2.89	6.67±2.89	3.33±2.89	3.33±2.89	3.33±2.89
With mycorrhiza	73.33±2.89	75.00±5.00	83.33±2.89	78.33±2.89	76.67±2.89	78.33±2.89

combined with mycorrhizal inoculation significantly increased the grafting success rate (Table 2). When the soil was inoculated with mycorrhiza, the application of BAP produced the highest grafting success rate (93.33%), whereas in the absence of mycorrhiza, the success rate was 80%. Meanwhile, IBA, coconut water, bean sprout extract, and shallot extract showed lower success rates. Thus, the grafting success was influenced by the combination of mycorrhizae and the type of plant growth-promoting substance, with BAP having the most optimal effect.

3.3 Time of Apical Bud Emergence

The results of the study showed that there was a significant interaction between mycorrhizal inoculation and plant growth-promoting substances on the time of apical bud emergence. The data presented in Table 3 indicate that the application of growth-promoting substances along with mycorrhizal inoculation significantly accelerated apical shoot growth. The fastest shoot formation was achieved in mycorrhizal seedlings treated with BAP or coconut water (22.67 days after grafting). BAP also accelerated shoot formation in

seedlings without mycorrhizal inoculation (24.5 days after grafting). However, no significant differences were observed among the effects of the various growth-promoting substances tested, either with or without mycorrhizal inoculation. In both the presence and absence of growth-promoting substance factor, *Liberica* coffee seedlings inoculated with mycorrhizae consistently showed faster shoot growth than those without mycorrhizae.

3.4 Apical Shoot Diameter

Data showed that there was a significant interaction between mycorrhizal inoculation and plant growth-promoting substances on apical shoot diameter. As shown in Table 4, mycorrhizal inoculation significantly increased the apical shoot diameter of *Liberica* coffee seedlings, whether or not growth-promoting substances were present. However, in the absence of growth-promoting substances, the diameter decreased significantly, even when the growth medium was inoculated with mycorrhizae. The application of BAP produced the greatest shoot diameter in the presence of mycorrhizae (3.80 mm) and in the absence of mycorrhizae (3.00 mm).

Table 2: Effects of mycorrhizal inoculation and the application of growth-promoting substances on the success rate of *Liberica* coffee grafting (%).

Mycorrhizal inoculation	Control	IBA	BAP	Coconut water	Bean sprout extract	Shallot extract
Without mycorrhiza	66.67±2.89 d B	75.00±0.00 b B	80.00±0.00 a B	70.00±0.00 c B	65.00±0.00 d B	70.00±0.00 c B
With mycorrhiza	75.00±0.00 d A	81.67±2.89 bc A	93.33± 2.89 a A	83.33±2.89 b A	80.00±0.00 c A	81.67±2.89 bc A

Notes: values followed by the same lowercase letter in the same row and the same uppercase letter in the same column are not significantly different ($LSD_{0.05} = 3.27$).

Table 3: Effects of mycorrhizal inoculation and the application of growth-related substances on the time of apical bud emergence (days after grafting).

Mycorrhizal inoculation	Control	IBA	BAP	Coconut water	Bean sprout extract	Shallot extract
Without mycorrhiza	33.83±0.29 a A	26.83±0.29 b A	24.50±0.50 e A	25.00±0.00 d A	25.67±0.29 c A	25.00±0.00 d A
With mycorrhiza	28.00±0.00 a B	25.33±0.58 b B	22.67±0.58 e B	22.67±0.58 e B	24.33±0.58 c B	23.67±0.58 d B

Notes: values followed by the same lowercase letter in the same row and the same uppercase letter in the same column are not significantly different ($LSD_{0.05} = 0.65$).

Table 4: Effects of mycorrhizal inoculation and the application of growth-related substances on the diameter of the apical shoot (mm).

Mycorrhizal inoculation	Control	IBA	BAP	Coconut water	Bean sprout extract	Shallot extract
Without mycorrhiza	2.52±0.03 c B	2.75±0.00 b B	3.00±0.00 a B	2.82±0.08 b B	2.75±0.01 bc B	2.78±0.03 b B
With mycorrhiza	2.82±0.03 e A	2.92± 0.10 d A	3.80± 0.10 a A	3.23±0.06 b A	3.13±0.12 c A	2.93±0.12 d A

Notes: values followed by the same lowercase letter in the same row and the same uppercase letter in the same column are not significantly different ($LSD_{0.05} = 0.10$).

3.5 Total Leaf Area

The results of the study showed that total leaf area was significantly influenced by the interaction between mycorrhizal inoculation and plant growth-promoting substances application. As shown in Table 5, mycorrhizal inoculation significantly increased total leaf area, particularly when combined with BAP, coconut water or bean sprout extract. BAP application produced the greatest total leaf area in the presence of mycorrhizae (72.00 mm²) and in the absence of mycorrhizae (60.33 mm²). Conversely, in the absence of growth-promoting substances, the total leaf area was the lowest in both groups of seedlings.

3.6 P Absorption

A significant interaction between mycorrhiza and plant growth-promoting substances was found in phosphorus absorption. In this study, seedlings grown in peat soil without mycorrhizal inoculation and without growth-promoting substance treatment had significantly lower phosphorus uptake than those grown in mycorrhizal-inoculated media and treated with growth-promoting substances, except in the comparison of those without growth-promoting substance and those treated with shallot extract (Table 6). The use of BAP significantly increased phosphorus uptake in both mycorrhizal-inoculated (36.60) and non-mycorrhizal-inoculated (25.60) seedlings.

3.7 Seedling Quality Index

There was a significant interaction between mycorrhizae and plant growth-promoting substances on seedling quality index is indicated in Table 7. Liberica coffee seedlings inoculated with mycorrhizae had a higher seedling quality index than those without mycorrhizae across all the growth-promoting substances. Furthermore, compared with seedlings treated with all types of growth-promoting substances, both mycorrhizal and nonmycorrhizal seedlings grown in media without growth-promoting substances had the lowest seedling quality index. The highest quality index occurred in seedlings treated with BAP whether they were grown in media supplemented with mycorrhizae (0.84) or without mycorrhizae (0.71); their quality indices were significantly higher than those of the seedlings treated with the other growth-promoting substances.

4 DISCUSSION

Mycorrhizal inoculation of the peat soil growth medium resulted in excellent improvements in the growth of Liberica coffee seedlings, as supported by approximately 77.5% root infection occurring in the mycorrhizal treatment. These conditions demonstrated the natural formation of an association between mycorrhizae and plant roots, without

Table 5: Effects of mycorrhizal inoculation and the application of growth-promoting substances on the total leaf area (mm²).

Mycorrhizal inoculation	Control	IBA	BAP	Coconut water	Bean sprout extract	Shallot extract
Without mycorrhiza	35.40±6.39 c A	46.33±0.58 b A	60.33±0.58 a B	44.13±8.19 bc B	38.27±0.80 bc B	45.67±0.58 b A
With mycorrhiza	39.00±1.00 c A	51.70±0.58 b A	72.00±1.00 a A	57.00±2.65 b A	48.59±0.53 bc A	51.03±0.58 b A

Notes: values followed by the same lowercase letter in the same row and the same uppercase letter in the same column are not significantly different (LSD_{0.05} = 5.44).

Table 6: Effects of mycorrhizal inoculation and the application of growth-related substances on P absorption (mg per seedling).

Mycorrhizal inoculation	Control	IBA	BAP	Coconut water	Bean sprout extract	Shallot extract
Without mycorrhiza	14.56±2.11 d A	19.93±0.38 bc B	25.60±0.30a B	21.58±1.01 ab B	15.13±0.32 cd B	22.67±0.46 ab A
With mycorrhiza	16.19±0.50 d A	27.81±1.12 b A	36.60±9.91 a A	27.63±1.24 bc A	22.40±0.97 c A	26.79±0.99 bc A

Notes: values followed by the same lowercase letter in the same row and the same uppercase letter in the same column are not significantly different (LSD_{0.05} = 5.14).

Table 7: Effects of mycorrhizal inoculation and the application of growth-promoting substances on the seedling quality index.

Mycorrhizal inoculation	Control	IBA	BAP	Coconut water	Bean sprout extract	Shallot extract
Without mycorrhiza	0.33±0.01 f B	0.65±0.02 b B	0.71±0.01 a B	0.63±0.02 c B	0.42±0.01 e B	0.47±0.02 d B
With mycorrhiza	0.47±0.01 e A	0.73±0.01 b A	0.84±0.02 a A	0.75±0.01 a A	0.52±0.02 d A	0.57±0.02 c A

Notes: values followed by the same lowercase letter in the same row and the same uppercase letter in the same column are not significantly different (LSD_{0.05} = 2.35).

being affected by the application of plant growth-promoting substances. Moreover, seedlings without mycorrhizae also showed infection, even when the growth medium had been sterilized. This is likely due to initial contamination of the growth medium before the seedlings were transferred to larger polybags. In addition, seedlings originating from environments infected with mycorrhizae might carry mycorrhizal spores or hyphae that adhere to root tissue. These spores and hyphae perhaps remain viable and develop in new sterile medium. This finding is supported by the study of (Campo et al., 2020), who showed that mycorrhizal spores and hyphae that survive on plant roots are able to survive under sterilized media conditions, such that mycorrhizal infection can still occur even when the growth medium is sterilized. This reflects the adaptive ability of mycorrhizae to maintain survival and re-establish associations under favorable conditions.

In general, the application of plant growth-promoting substances, particularly BAP, significantly increased the success of grafting and the vegetative growth of Liberica coffee seedlings. The application of BAP to scions during grafting might accelerate bud emergence, increase apical shoot diameter, and increase total leaf area in both mycorrhizal and non-mycorrhizal-inoculated seedlings. This indicates that exogenous cytokinins stimulate meristematic activity and tissue formation post grafting phase.

Local application of BAP at the graft point effectively accelerated tissue fusion between the rootstock and scion during Liberica coffee grafting. As a synthetic cytokinin, BAP plays a key role in stimulating meristematic cell division and tissue differentiation, which are the initial stages of callus formation and vascularization in the graft area. Rasool et al. (2020) suggested that cytokinins such as BAP promote the expression of genes involved in tissue regeneration and vascular tissue formation, thereby accelerating physiological integration between the scion and rootstock. Similar findings reported by Feng et al. (2024) revealed that the application of BAP at the graft point might optimize local hormonal signals that support wound healing by increasing cell proliferation and metabolic activity. This is consistent with a report by (Jogaiah & Porika, 2023), who showed that soaking scions in a BAP solution (100 ppm) before grafting can increase grafting success by up to 68%. This increase was associated with the activity of antioxidant enzymes, such as peroxidase and polyphenol oxidase, which play roles in callus formation and vascular tissue connectivity. These findings suggest that BAP application not only accelerates tissue union but also improves graft quality by regulating physiological and biochemical processes at the graft site.

Compared with that without mycorrhizae, the success of grafting of Liberica coffee seedlings was greater when all the growth-promoting substances were applied in the presence of mycorrhizae. This finding indicates a synergistic effect of

mycorrhizae and growth-promoting substances in supporting the graft union process. Mycorrhizae play a crucial role in enhancing the effectiveness of growth-promoting substances by increasing the uptake of key nutrients such as phosphorus and nitrogen (Ashwin et al., 2018; Jiang et al., 2021; Lu et al., 2023; Wu et al., 2024) and by stimulating the production of endogenous hormones such as auxin and cytokinin, which support cell division and callus formation (Begum et al., 2019). These improved physiological conditions increase plant sensitivity to natural and synthetic growth regulators, such as BAP, thereby optimizing vascularization and tissue regeneration at the grafting point. These findings are consistent with Gao et al. (2023), who reported that the combination of mycorrhizae and plant hormones accelerated tissue regeneration in vegetatively propagated plants.

Our study revealed that, compared with those grown on non-inoculated soil, those treated with growth-promoting substances and grown on mycorrhizal-inoculated soil exhibited faster shoot growth. This occurred because mycorrhizae promoted cell division and shoot activation by increasing the uptake of nutrients such as phosphorus, nitrogen, and potassium (Wu et al., 2024), as well as by stimulating endogenous hormones such as auxin and cytokinin (Gao et al., 2023). This combination of increased nutritional and hormonal activity enhances plant metabolism after grafting, thereby accelerating shoot growth and tissue recovery.

This study also revealed that, in the absence of growth-promoting substances, seedlings took longer to develop apical shoots, whether in media with or without mycorrhizal inoculation. This is probably because graft tissue regeneration depends on hormonal factors and nutrient availability, resulting in slower callus and vascular tissue formation in their absence. Köse and Güleriyüz (2006) and Gao et al. (2023) suggested that the application of exogenous hormones such as cytokinins or auxins accelerates callus formation and vascularization. In contrast, without hormonal treatment, the healing process and shoot growth take longer. Although mycorrhizae improve plant physiological status, the presence of growth-promoting substances is still necessary to trigger cell regeneration signals in the early phase of grafting.

The increase in apical shoot diameter in response to BAP application can be explained by the role of cytokinins in activating cell division and expansion in shoot meristem tissue. BAP applied to the graft area can translocate acropetally to the apical shoot and induce cell division activity in the meristematic zone (Cortleven et al., 2019; Zhai et al., 2021). Cytokinins also play a role in accelerating callus and vascular tissue formation, strengthening connectivity between the rootstock and scion, and increasing nutrient and hormonal flow to the growing point (Gao et al., 2023; Kieber & Schaller, 2018).

Apical shoot diameter was greater in mycorrhizal-inoculated seedlings than in non-mycorrhizal-inoculated

seedlings for all the growth-promoting substances applied. Mycorrhizae improve the efficiency of nutrient uptake, particularly of phosphorus and nitrogen, which play roles in cell division and shoot meristem enlargement (Deja-Sikora et al., 2020; Kartika & Gusniwati, 2019; Ramayana et al., 2022). In addition, mycorrhizal associations increase the activity of antioxidant enzymes and the production of endogenous plant hormones such as auxin and cytokinin, which promote shoot growth and accelerate the recovery of connective tissue (Wu et al., 2022). Therefore, compared with nonmycorrhizal seedlings, mycorrhizal seedlings grew more vigorously and presented greater shoot growth capacity.

The most minor shoot diameters were detected in seedlings without the application of growth-promoting substances, indicating that exogenous hormonal signals are required to activate cell division and tissue differentiation in the early stage of grafting (Cortleven et al., 2019; Zhai et al., 2021). Hormones such as BAP and IBA play direct roles in stimulating callus formation and vascularization. At the same time, effectiveness of mycorrhizae in supporting shoot growth is enhanced when these compounds are combined with exogenous hormones.

BAP, also known as N6-benzyladenine, functions as a synthetic cytokinin that mimics the activity of natural cytokinins in regulating cell division, tissue differentiation, and leaf expansion (Aroca et al., 2013; Rehman et al., 2023; Selim et al., 2023). The increase in leaf area upon BAP application indicates a high photosynthetic capacity in the seedlings, which plays a crucial role in supporting post grafting growth. Moreover, mycorrhizae enhanced the plant's response to BAP by increasing the efficiency of nutrient uptake, particularly that of phosphorus, which plays a role in energy metabolism and the biosynthesis of growth compounds. Goh et al. (2019) reported that plants inoculated with mycorrhizae and treated with synthetic cytokinins exhibited higher levels of colonization and physiological activity than those without mycorrhizae did, indicating a positive relationship between mycorrhizal colonization and cytokinin activity.

The synergistic interaction between BAP and mycorrhizae creates physiological conditions that support vegetative growth, including leaf expansion and increased nutrient uptake. Cytokinins such as BAP not only stimulate cell division but also enhance physiological efficiency in nutrient absorption, increasing the expression of nutrient transporter genes and lateral root activity (Cortleven et al., 2019). In seedlings without mycorrhizae, BAP application can compensate for the limited nutrient uptake caused by the absence of external hyphae. In contrast, in seedlings with mycorrhizae, BAP synergizes with mycorrhizal hyphae, expanding the nutrient-uptake area and facilitating phosphorus transport to plant tissues. This interaction results in significantly increased P uptake efficiency and increased vegetative growth.

The increase in total leaf area in mycorrhizal-inoculated seedlings treated with BAP, coconut water, or bean sprout extract indicated a synergistic effect between mycorrhizae and growth-promoting substances on the expansion of the plant's photosynthetic area. Mycorrhizae increase phosphorus and nitrogen uptake (Aishwarya et al., 2022; Ramayana et al., 2022), which are needed for leaf tissue formation and chlorophyll synthesis, whereas growth-promoting substances increase cell expansion through hormonal regulation (Goh et al., 2019; Zhai et al., 2021). However, when IBA or shallot extract was applied, the increase in total leaf area was relatively small because these hormones play important roles in root and callus formation (Gao et al., 2023).

Furthermore, the use of natural growth-promoting substances such as coconut water also had a significant effect on mycorrhizal-inoculated seedlings. However, the effect was weaker than that of synthetic growth-promoting substances. These findings indicated that the synergistic contribution of mycorrhizae and growth-promoting substances promoted shoot formation and leaf expansion. Coconut water contains high levels of natural phytohormones such as cytokinins, gibberellins and auxins, which can directly accelerate cell division, shoot growth, and tissue regeneration (Smith & Read, 2017). The combination of mycorrhizal inoculation and coconut water application created an optimal physiological environment, in which the increased nutrition provided by mycorrhizae enhanced the effectiveness of the phytohormones in coconut water, significantly accelerating bud emergence. Thus, the synergy between mycorrhizae, synthetic growth-promoting substance (BAP), and natural phytohormones in coconut water collectively enhanced the physiological efficiency of plants in accelerating shoot growth in grafted Liberica coffee seedlings.

These improvements in growth parameters, as previously mentioned, have direct implications for the physiological quality of the seedlings, as reflected by the highest seedling quality index in the application of BAP, either with or without mycorrhizal inoculation. These findings indicated that BAP was effective at stimulating meristematic activity, cell division, and tissue expansion and increasing vegetative growth, which overall were integrated into the seedling quality index (Smith & Read, 2017; Zhai et al., 2021). These findings confirm that the application of synthetic cytokinins such as BAP might optimize vegetative growth and accelerate the recovery of grafted tissue. On the other hand, coconut water contains various natural phytohormones, such as auxins, cytokinins, and gibberellins, which can also stimulate shoot and root growth and accelerate the recovery of grafted tissue (Kartika et al., 2023). The combination of mycorrhizae with natural plant growth regulators, such as coconut water, creates a synergistic effect that improves seedling quality, as reflected by high seedling quality index values.

Compared with that in non-mycorrhizal-inoculated seedlings, the quality index in mycorrhizal-inoculated seedlings was greater across all the applied growth-promoting substances. These findings confirm the role of mycorrhizae in enhancing nutrient uptake and hormonal balance that support vegetative growth. Mycorrhizae also increase the activity of antioxidant enzymes and the production of endogenous hormones, such as cytokinins and auxins, that contribute to biomass formation and leaf area (Wu et al., 2022). Moreover, in the absence of growth-promoting substances, the seedling quality index was lowest because exogenous hormonal stimuli were absent, which accelerated physiological and morphogenetic activities in the plants. Growth-promoting substances such as BAP and IBA accelerate the formation of vascular tissue and increase the distribution of photosynthetic products to actively growing tissues (Cortleven et al., 2019; Taiz et al., 2015). The application of these hormones could increase the height, stem diameter, and dry weight of grafted Robusta coffee seedlings (Kamilah et al., 2024). Thus, the low seedling quality index in the absence of growth-promoting substances was due to limited hormonal signals that regulate cell division and tissue differentiation, resulting in slow vegetative growth despite the mycorrhizal association.

5 CONCLUSIONS

The application of plant growth-promoting substances and mycorrhizal inoculation were critical for improving the graft union, growth and quality of Liberica coffee seedlings grown in peat soil. The best performance was obtained when the grafts were treated with the synthetic growth-promoting substance BAP and grown on mycorrhizal-inoculated soil. Natural growth-promoting substances such as coconut water, bean sprout extract, and shallot extract also contributed positively, but their effects were weaker than those of synthetic growth-promoting substances such as BAP and IBA.

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7 AUTHORS' CONTRIBUTIONS

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8 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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