



Research article

Evaluation of mycorrhizal application in overcoming soil water limitations in cocoa seedlings

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Abstract

Importance of the work: Mycorrhiza could play a major role in overcoming soil water limitations that restrict crop growth.

Objectives: To evaluate the impact of mycorrhizal application on enhancing cocoa seedling resilience to water limitations in the soil.

Materials and Methods: The experiment was conducted using a split-plot design based on a randomized block design. The main plot had two soil moisture levels: 100% and 50% field capacity. The sub-plots were applications of different mycorrhizae: M0, CM, CGK1, CGK2, CGK3, CGK4, CGK5, CS1, CS2, ACS1, ACS2, ACS3 and RCS.

Results: The 12 mycorrhizae evaluated had different responses to soil moisture levels. The soil moisture level at 50% field capacity in the control treatment significantly reduced the nitrogen uptake, seedling height, shoot fresh weight and shoot dry weight of cocoa seedlings; however, it did not cause drought stress. These four variables served as indicators for evaluating mycorrhizal applications in managing soil moisture levels at 50% field capacity. Consistently, the mycorrhizae CGK3, ACS3 and RCS exhibited similar performance at both 50% and 100% field capacity across these four indicator variables.

Main finding: The identification of three mycorrhizae, CGK3, ACS3 and RCS, could enhance the diversity of mycorrhizae that help cocoa seedlings overcome soil water limitations.

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Introduction

Cocoa (*Theobroma cacao* L.) is grown in a wide range of soil conditions throughout Indonesia (Rubiyo and Siswanto, 2012). There are numerous challenges in developing these plants due to their susceptibility to abiotic stresses, including water limitation and low soil fertility. Other studies have shown that cocoa growth and production are also vulnerable to climate change, including shifts in rainfall distribution and high temperatures (Santosa et al., 2018; Lahive et al., 2019). Cocoa plantations in Indonesia are facing water shortages, particularly in arid regions such as East Nusa Tenggara. In addition, Indonesia had experienced long dry periods due to the El Niño phenomenon, resulting in a 62% decrease in cocoa productivity and farmer income (Keil et al., 2008).

Water limitations can cause drought stress in cocoa plants, which are a drought-sensitive crop (Carr and Lockwood, 2011). The effect of drought stress depends on the growth stage, the level and duration of stress and the cocoa cultivar. For example, the early phase of the cocoa plant is more susceptible to drought than the mature plant (Kaba et al., 2021). Drought stress will inhibit photosynthetic activity and plant metabolism, resulting in the inhibition of leaf and stem development, wilted plants and the shedding of leaves (Carr and Lockwood, 2011; Zakariyya and Indradewa, 2018) and decreased production and plant death (Keil et al., 2008; Gateau-Rey et al., 2018). Mitigating the effects of drought can be carried out to sustain crop productivity and farmer income. Drought mitigation efforts include: developing tolerant plants (Zasari et al., 2020; Juby et al., 2021); shading management (Abdulai et al., 2018; Famuwagun and Agele, 2019); irrigation techniques (Carr and Lockwood, 2011; Famuwagun et al., 2017); ameliorants (Ali et al., 2017; Dariah et al., 2021); and potassium (K) fertilization and arbuscular mycorrhiza use (Kaba et al., 2021). Enriching mycorrhizal fungi in the plant rhizosphere is a technology designed to enhance plant resistance to drought and several researchers have explored this approach. For example, Katoda et al. (2025) indicated that mycorrhizae increased the efficiency of water and nutrient uptake, while also improving resistance to drought stress. For corn plants experiencing drought stress (with soil moisture at 40% field capacity), it is recommended to use 20 g of mycorrhizae per plant. In addition, other studies showed that the different types of mycorrhizae had varying effectiveness, depending on soil moisture levels (Shukla et al., 2013; Deepika and Kothamasi, 2015). Furthermore,

Shao et al. (2023) concluded that mycorrhizae play an optimal role when supported by appropriate water and phosphorus (P) conditions.

Arbuscular mycorrhizal fungi (AMF) are a group of obligate symbiont fungi that form mutually beneficial relationships with about 80% of all plant species (Berruti et al., 2016). AMF play a crucial role in helping plants reduce drought stress, both directly and indirectly. Mycorrhizal colonization on plant roots forms intraradical and extraradical hyphae; extraradical hyphae modify root architecture, expanding the absorption area for increased water and nutrient uptake (Bahadur et al., 2019). Mycorrhizal glomalin production and hyphae enhance soil structure and aggregation, also increasing the water storage capacity (Rillig and Mummey, 2006). The association of mycorrhizae with plant roots promotes growth: by enhancing nutrient accumulation mechanisms, by improving the root absorption of nutrients, including N, P, K, Ca, Mg, Zn, Cu, Mn and Fe (Al-Karaki and Al-Raddad, 1997; Gholamhoseini et al., 2013; Begum et al., 2019; David et al., 2021); releasing growth-promoting hormones (Liao et al., 2018; Pons et al., 2020); and increasing plant disease resistance and tolerance to other abiotic stresses such as high salinity, high heavy metal content and extreme temperatures (Tchameni et al., 2012; Berruti et al., 2016; Begum et al., 2019). Other studies have shown that inoculating cocoa seedlings with mycorrhizal fungi promoted seedling growth (Tchameni et al., 2012; Rokhmah et al., 2020). However, further study is needed to explore the effects of mycorrhizae and their interaction with soil moisture levels on cocoa seedlings. The water requirements of young cocoa at the nursery level differ from those of mature cocoa in the field, highlighting the importance of understanding soil moisture levels for cocoa during the nursery stage and the role of mycorrhizae in reducing water stress. On the other hand, the type and performance of mycorrhizae found in nature are highly diverse, allowing for compatibility between mycorrhizal species and their host plants under varying soil water conditions. The current study aimed to evaluate the impact of mycorrhizal application on enhancing cocoa seedling resilience to water limitations in the soil.

Materials and Methods

The experiment was conducted in the Greenhouse and Laboratory of the Indonesian Industrial and Beverage Crops Research Institute in Pakuwon, Sukabumi, West Java Province, Indonesia. The greenhouse roof was covered with shade netting

that allowed 35% sunlight penetration to provide optimal sunlight for the cocoa seedlings. In the greenhouse, daily temperatures were in the range 22.1–33.9°C, with an average of 27.4°C and humidity was in the range 48–99%, with an average of 82%. The experimental materials used included cocoa seeds from the Sulawesi 1 clone, non-sterilized Ultisol soil sourced from Jasinga, Bogor, West Java, Indonesia (Table 1), sand, corn seeds, a mycorrhizal consortium and commercial mycorrhizae.

Table 1 Unsterilized soil properties used

pH	Organic C (%)	N (%)	P (mg/kg)	Exchangeable cations (cmol(+)/kg)			CEC
				K ⁺	Ca ²⁺	Mg ²⁺	
4.11	2.25	0.34	1.77	0.33	2.79	1.54	34.4

CEC = cation exchange capacity.

Experimental design

The experiment was conducted using a split-plot design based on a randomized block design with three replications. The main plot focused on two soil moisture levels (SMLs): 100% field capacity (FC) and 50% FC. The subplots were mycorrhizal application (MA) treatments, consisting of M0 (the control, with no added mycorrhizae), CM (commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT)), cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia (CGK1, CGK2, CGK3,

CGK4, CGK5), cocoa sourced from Sukabumi City in West Java Province, Indonesia (CS1, CS2), Arabica coffee sourced from Sukabumi City (ACS1, ACS2, ACS3) and from robusta coffee sourced from Sukabumi City (RCS), as shown in Table 2.

Procedure for mycorrhizal culture and cocoa seeding

The mycorrhizae utilized in the current study originated from Gunung Kidul (Yogyakarta) and Sukabumi (West Java). They were maintained using corn (*Zea mays* L.) as the host plant, grown in a mixture of 3 kg of a sand-to-soil ratio of 2:1 for 3.5 mth. Initially, the planting media were sterilized in an autoclave at 121°C for 15 min and then used to fill 3 kg plastic pots. Before planting, the corn seeds were soaked in 1% NaOCl for 5 min and then rinsed three times with distilled water. Afterward, the mycorrhizal inoculum was placed near the corn seeds and maintained until germination occurred. Afterward, the corn seedlings were maintained for 3.5 mth and fertilized with an NPK compound fertilizer (20-20-20). This process involved diluting 5 g of fertilizer in 1 L of water and applying 50 mL to each plant every 2 wk. After 2.5 mth without water, the plants dried out. Subsequently, root cuttings mixed with dry soil media were used as the source of the mycorrhizal inoculum. The identification of the mycorrhizal culture type was based on spore shape, following the guidelines established by Brundrett et al. (1996). The range of mycorrhizal populations used in the experiment was 33–250 spores per 50 g of carrier.

Table 2 Different types of mycorrhizae used.

Type of mycorrhiza	Source of mycorrhiza (vegetation, location)	Genus and species level identification	Spore number per 50 g of carrier
CM	BPPT	<i>Gigaspora margarita</i> , <i>Glomus manihotis</i> , <i>Acaulospora</i> sp.	250
CGK1	Cocoa, Gunung Kidul 1	<i>Scutellospora</i> sp.	76
CGK2	Cocoa, Gunung Kidul 2	<i>Scutellospora</i> sp.	74
CGK3	Cocoa, Gunung Kidul 3	<i>Scutellospora</i> sp., <i>Glomus</i> sp.	37
CGK4	Cocoa, Gunung Kidul 4	<i>Scutellospora</i> sp., <i>Glomus</i> sp., <i>Gigaspora</i> sp.	40
CGK5	Cocoa, Gunung Kidul 5	<i>Glomus</i> sp.	33
CS1	Cocoa, Sukabumi 1	<i>Scutellospora</i> sp., <i>Gigaspora</i> sp.	28
CS2	Cocoa, Sukabumi 2	<i>Acaulospora</i> sp.	38
ACS1	Arabica coffee, Sukabumi 1	<i>Scutellospora</i> sp.	62
ACS2	Arabica coffee, Sukabumi 2	<i>Glomus</i> sp.	34
ACS3	Arabica coffee, Sukabumi 3	<i>Glomus</i> sp.	35
RCS	Robusta coffee, Sukabumi	<i>Glomus</i> sp.	21

M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City.

The planting media for the cocoa seedlings consisted of 3 kg of dry soil packed into polybags measuring 30 cm × 16 cm × 16 cm. The cocoa seeds were soaked in a 1% sodium hypochlorite (NaOCl) solution for 5 min and then rinsed three times with distilled water. Next, 50 g of mycorrhizae were placed in each planting hole in the pots and the cocoa seeds were added and covered with soil. The SML treatments were conducted 2 mth after planting (MAP). The SML was determined based on soil FC analysis, using 57.5% dry weight at a potential freely of 2.54, with 16% for air-dry soil. This analysis helped in determining irrigation water requirements at both 100% FC and 50% FC. Watering was undertaken every 2 d.

Data collection

Observations and analyses conducted on the cocoa seedlings at 5 MAP consisted of percentage of root infection by mycorrhizae, N and P contents in the shoot, N and P uptake levels by the shoot, available P in the soil, seedling height, stem diameter, leaf number, leaf area, root volume, root length, shoot fresh and dry weights and root fresh and dry weights.

Percentage of root infection by mycorrhiza

Root infection by mycorrhizae was determined using fresh and fine root samples from the cocoa seedlings. A staining technique was applied, as suggested by Phillips and Hayman (1970). Root samples were washed and cut approximately 2 cm from the tip. The samples were soaked for 24 hr in a glass bottle containing a 10% KOH solution, then rinsed with running water. Next, the samples were placed back into the glass bottle and soaked in a 2% HCl solution for 10 min. Then, a 0.05% Trypan Blue dye solution was added and left for 24 hr to achieve optimal staining. In each replication, 50 root sections were examined, with 10 root sections per slide. Root infection was determined using a binocular microscope, where infected roots had vesicles and arbuscules within the cortex tissue. The percentage of root infection was measured using Eq. (1):

$$\text{Percentage of root infection} = \frac{\text{(Number of infected roots)}}{\text{(Number of all roots observed)}} \times 100 \quad (1)$$

The root infection rate was categorized into five classes: very low (0–5%), low (6–25%), moderate (26–50%), high (51–75%) and very high (76–100%).

Shoot N and P contents, shoot N and P uptake and soil available P

A shoot sample (0.5 g) was used to analyze the N and P contents in the shoot. Each sample was oven-dried at 70 °C before grinding. Then, the N content in the shoot sample was analyzed based on digesting with sulfuric acid (H₂SO₄) and then using the Kjeldahl method (Motsara and Roy, 2008). The P content in the shoot sample was measured based on digesting with perchloric acid (HClO₄) and nitric acid (HNO₃), followed by analysis using a spectrophotometer and a flame photometer (Motsara and Roy, 2008). Notably, the N and P uptake in the shoot was determined based on multiplying their respective content by the shoot dry weight. In contrast, the available P in the soil was extracted using the Bray 1 method and quantified using a spectrophotometer (Food and Agriculture Organization of the United Nations, 2021).

Cocoa root and leaf measurement

The root length was measured using a measuring tape. The root volume was calculated by measuring the change in water level in a 500 mL measuring cylinder after immersing the roots in the water. Additionally, leaf area was measured using the method described by Salazar et al. (2018), as shown in Eq. (2):

$$LA = 9.634 + 0.2022P - 3.3458L + 0.0985P^2 + 1.2179L^2 \quad (2)$$

where LA is the leaf area (measured in square centimeters) and Land P are the maximum leaf width and maximum leaf length, respectively (both measured in centimeters).

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range tests at the 5% level. This data analysis used the STAR (Statistical Tool for Agricultural Research) version 2.0.1 from IRRI (International Rice Research Institute), Philippines, (Azrai et al, 2017).

Results and Discussion

Effects of SML and MA treatments on soil phosphorus, nutrient uptake, and seedling growth

Based on the ANOVA results (Table 3), the only significant interaction between the SML and MA treatments was for available P in the soil. The SML treatment had a significant effect on root infection, N and P uptake by the leaves, seedling height, stem diameter, leaf number, root length, shoot fresh weight, shoot dry weight and root dry weight. On the other hand, MA significantly affected root infection, N and P uptake by leaves, available P in the soil, seedling height, leaf number, root volume, shoot dry weight and root dry weight.

The lack of a significant interaction between SML and MA for most observed variables indicated that the two treatment factors were independent (did not influence each other). This may have been due not achieving drought stress in the cocoa seedlings treated with 50% FC, thus nullifying any role of the mycorrhizal plant in managing drought stress. In this case, SML at 50% FC did not fall into the category of drought stress for cocoa seedlings, which can lead to leaf shedding, seedling wilt and death (Carr and Lockwood, 2011; Zakariyya and Indradewa, 2018); instead it only limits growth. This was consistent with one of the plant responses to water deficit being drought escape, including reducing the dehydration process by limiting growth (Fang and Xiong, 2015). In this regard, drought stress may occur if the SML is below 50% FC. Other studies have shown that the effect of mycorrhizae is highly dependent on soil moisture levels and plant species

(Shukla et al., 2010), particularly soil hydraulic properties, which are dynamic factors in assessing the water relations of mycorrhizal plants (Pauwels et al., 2023). In *Lallemantia* sp., mycorrhizae had a significant impact under three drought stress conditions, specifically at soil available-water depletion levels of 30%, 60% and 90% (Paravar et al., 2024). In maize plants, the significant effect was noted at a 40% soil moisture level (Katoda et al., 2025). Furthermore, in *Phaseolus mungo*, *Triticum aestivum*, *Eucalyptus tereticornis* and *Albizia procera*, mycorrhizae were significant at soil moisture levels of 8, 16 and 32% FC, respectively, (Shukla et al., 2010), as well as for these plants in agroforestry systems (Shukla et al., 2013).

Since there was generally no interaction between the two factors for most of the tested variables, the next analysis examined the SML in each MA, as well as the MA in each SML, separately for each variable, except for the available P, because the ANOVA results indicated a significant interaction. However, in accordance with the objectives of the current study, the focus was on comparisons between SML (100% FC and 50% FC) for M0 and for each of the 12 mycorrhizal types. Thus, if, for example, the test results for M0 indicated 100% FC were significantly better than 50% FC for a particular variable; then this variable could be used as an indicator to evaluate the ability of each mycorrhizal type to overcome water limitations at 50% FC and conversely, if the result were not significant. This approach was valid because the experiment used a split-plot design, with the two main plots installed separately. Analyzing data on both factors (SML and MA) simultaneously could obscure the specific effects of each factor. Nevertheless, these simultaneous analyses were conducted as well, to investigate the simple effect of SML and MA for comparison.

Table 3 Analysis of variance results for 16 observed variables

No.	Variable	Soil moisture level (SML)	Mycorrhizal application (MA)	SML × MA
1	Root infection	*	**	ns
2	N content in the shoot	ns	ns	ns
3	P content in the shoot	ns	ns	ns
4	N uptake by the shoot	*	*	ns
5	P uptake by the shoot	*	**	ns
6	Available P in the soil	ns	*	*
7	Seedling height	*	**	ns
8	Stem diameter	**	ns	ns
9	Number of leaves	*	*	ns
10	Leaf area	ns	ns	ns
11	Root volume	ns	*	ns
12	Root length	*	ns	ns
13	Shoot fresh weight	**	ns	ns
14	Shoot dry weight	**	**	ns
15	Root fresh weight	ns	ns	ns
16	Root dry weight	**	*	ns

* $p < 0.05$; ** $p < 0.01$; ns = non-significant.

Interaction effect of soil moisture level and mycorrhizal application on soil available P

Based on the statistical analysis, the interaction between SML and MA significantly affected available P in the soil (Table 4). Nearly all MA treatments showed comparable levels of available P at 100% FC and 50% FC, except that CS1 and ACS3 were lower at 50% FC than at 100% FC, whereas CGK3 and CGK5 were lower at 100% FC than at 50% FC. Based further analysis, especially at SML 50% FC, several mycorrhizal types (CGK2, CGK3, CGK4, CGK5) significantly increased available P compared to the control. Furthermore, at SML 100% FC, available P in CS1, CS2 and ACS3 were higher than in the control. The positive role of mycorrhizae on P availability has been reported to depend on various factors such as the amount of compost added (Cavagnaro, 2014; Jan et al., 2014). Mycorrhizae could help to increase P availability if the P in soil would otherwise be difficult to utilize or inaccessible (Habte, 2000). Furthermore, the impact of mycorrhizae on increasing P availability could be affected by the presence of native mycorrhizae in the soil (Ortas, 2015).

On the other hand, the current analysis of the M0 treatment indicated that the available P at SML 100% FC did not differ significantly from that at 50% FC (Table 4). Consequently, the available P could not be used as an indicator of the ability of mycorrhizae to mitigate water limitations at 50% FC as was also the case for the other variables.

Effect of soil moisture level and mycorrhiza application on root

Further analysis showed that the root infection in the M0 treatment did not differ significantly between SML 100% and 50% FC. Therefore, the root infection variable could not be used as an indicator to assess the mitigation ability of mycorrhizae against SML at 50% FC (Table 5). Nevertheless, Püschel et al. (2021) showed that hyphal colonization of the substrate increased with decreasing soil moisture content, regardless of whether or not the plants were stunted. In contrast, He et al. (2020) reported that mycorrhizal root infection rates decreased under water stress conditions, while another study indicated that water stress did not influence the percentage of roots colonized by mycorrhizae (Morte et al., 2002). In the current study, despite no mycorrhizal inoculation in the control treatment, root infections still occurred, indicating the presence of native mycorrhizae in the soil, as the soil was not sterile. Berruti et al. (2016) argued that indigenous fungal populations in fields with unsterilized soil are relatively high, in contrast to sterile soil conditions. A greenhouse study conducted by Faye et al. (2020) using sterile soil showed varying levels of mycorrhizal colonization compared to field conditions, due to the presence of indigenous fungi in the unsterilized field soil.

Table 4 Interaction effect of soil moisture level and mycorrhizal application on available P in soil of cocoa seedlings at 5 mth after planting.

Type of mycorrhiza	Available P (mg/kg)		
	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
M0 (control)	4.25±1.22 ^{dA}	5.71±1.57 ^{cA}	4.98±0.95 ^d
CM	7.69±2.65 ^{bcd A}	9.18±0.59 ^{bcA}	8.43±1.26 ^{bc}
CGK1	5.32±0.45 ^{cd A}	10.97±3.98 ^{abcA}	8.14±2.19 ^c
CGK2	16.03±4.42 ^{a-d A}	16.50±4.03 ^{abA}	16.27±2.68 ^{ab}
CGK3	12.81±2.71 ^{a-d B}	16.09±2.79 ^{abA}	14.45±1.89 ^{abc}
CGK4	14.52±6.92 ^{a-d A}	19.71±2.17 ^{aA}	17.11±3.44 ^a
CGK5	12.10±4.81 ^{a-d B}	16.42±4.43 ^{abA}	14.26±3.08 ^{abc}
CS1	21.73±4.16 ^{aA}	4.78±0.11 ^{cB}	13.26±4.22 ^{abc}
CS2	17.84±5.10 ^{abc A}	12.31±4.76 ^{abcA}	15.07±3.35 ^{abc}
ACS1	17.13±4.33 ^{a-d A}	9.51±0.48 ^{bcA}	13.32±2.59 ^{abc}
ACS2	14.42±0.2 ^{a-d A}	8.91±1.30 ^{bcA}	11.67±1.37 ^{abc}
ACS3	18.99±3.26 ^{ab A}	4.55±0.62 ^{cB}	11.77±3.55 ^{abc}
RCS	11.74±0.63 ^{a-d A}	6.71±1.43 ^{bcA}	9.23±1.33 ^{abc}
Simple effect of soil moisture level	13.43±1.18 ^A	10.87±0.99 ^A	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City.

Mean ± SE within each column followed by different lowercase letters are significantly different (Duncan's test, $p < 0.05$), whereas different uppercase superscript letters indicate significant differences ($p < 0.05$) among means within each row.

Table 5 Effect of soil moisture level and mycorrhizal application on percentage of root infection in cocoa seedlings at 5 mth after planting.

Types of mycorrhiza	Root infection by mycorrhiza (%)		
	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
M0 (control)	21.67±6.17 ^{aA}	21.00±2.08 ^{dA}	21.33±2.92 ^d
CM	56.00±19.14 ^{aA}	62.00±19.43 ^{a-dA}	59.00±12.27 ^{bc}
CGK1	47.33±14.33 ^{aA}	60.33±18.22 ^{a-dA}	53.83±10.77 ^{bc}
CGK2	66.33±13.02 ^{aA}	51.67±20.48 ^{bcdA}	59.00±11.34 ^{bc}
CGK3	53.67±9.39 ^{aA}	94.00±0.00 ^{abA}	73.83±9.95 ^{ab}
CGK4	70.33±15.21 ^{aA}	74.67±17.68 ^{abcA}	72.50±10.47 ^{abc}
CGK5	68.00±12.70 ^{aA}	75.67±13.64 ^{abcA}	71.83±8.51 ^{abc}
CS1	55.33±14.62 ^{aA}	55.67±10.37 ^{a-dA}	55.50±8.02 ^{bc}
CS2	49.67±9.53 ^{aA}	55.67±21.61 ^{a-dA}	52.67±10.64 ^{bc}
ACS1	76.33±16.05 ^{aA}	96.67±3.33 ^{aA}	86.50±8.62 ^a
ACS2	44.67±8.01 ^{aA}	59.33±16.42 ^{a-dA}	52.00±8.81 ^{bc}
ACS3	38.00±10.02 ^{aA}	54.67±10.73 ^{bcdA}	46.33±7.55 ^c
RCS	58.33±18.85 ^{aA}	44.33±2.03 ^{cdA}	51.33±9.04 ^{bc}
Simple effect of soil moisture level	54.28±3.84 ^B	61.96±4.51 ^A	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City.

Mean ± SE within each column followed by different lowercase letters are significantly different (Duncan's test, $p < 0.05$), whereas different uppercase superscript letters indicate significant differences ($p < 0.05$) among means within each row.

Effect of soil moisture level and mycorrhiza application on shoot N and P contents

In the M0 treatment, there was no significant difference in the N and P contents due to the effects of SML 100% or 50% FC. Therefore, these two variables could not be used as indicator variables of mitigation against soil moisture limitation at 50% FC.

Effect of soil moisture level and mycorrhiza application on shoot N and P uptake

Further analysis revealed that the N uptake could serve as an indicator variable for mitigating against SML at 50% FC, unlike the P uptake. Based on the results, several mycorrhizal types (CGK2, CGK3, CGK5, CS2, ACS3, RCS) could effectively alleviate the water limitation of 50% FC (Table 7). Kapoor et al., 2020) revealed that the levels of soil moisture significantly affected the N uptake, since when plants undergo water stress, their ability to absorb N declines, with this reduction being associated with reduced photosynthetic activity and a smaller leaf area. Water stress can reduce N mineralization, thereby reducing the availability and uptake of N, which also results in a decrease in the rate of transpiration for N transport from the roots to the shoots (Tanguilig et al., 1987).

Based on the current results (Table 6), the average concentrations of N were below 2.5%, which is considered a low criterion for the canopy. This low concentration was likely due to the insufficient levels of available N, particularly in the absence of additional mineral fertilizers (Table 1). Mycorrhizal bio-stimulation can increase N uptake in cocoa grown in acidic soils, potentially reducing the need for inorganic fertilizers (Droh et al., 2016; Song and Finley, 2020). The processes involved in nutrient absorption from the soil and its release through mycorrhizal associations include the mobilization and acquisition of these nutrients by fungal mycelia. Furthermore, a transport mechanism exists for these absorbed nutrients at the interface between fungi and plant roots (Teotia et al., 2017).

Effect of soil moisture level and mycorrhiza application on cocoa seedling growth

Identical to the criteria in the previous analysis, the variables seedling height, shoot fresh weight and shoot dry weight served as effective indicators for mitigating water limitation at 50% FC. Based on these results, the mycorrhizal types CGK3, ACS3 and RCS consistently produced seedling height, shoot fresh weight and shoot dry weight values that were not significantly different between 100% and 50% FC (Tables 8-12). Therefore, these three mycorrhizal types could overcome the water limitations at 50% FC.

Table 6 Effect of soil moisture level and mycorrhizal application on shoot N contents of cocoa seedlings at 5 mth after planting.

Types of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
N content in the shoot (%)			
M0 (control)	1.99±0.04	2.05±0.18	2.02±0.08
CM	1.95±0.08	1.86±0.08	1.91±0.05
CGK1	1.88±0.15	2.23±0.18	2.06±0.13
CGK2	1.91±0.05	2.18±0.10	2.04±0.08
CGK3	1.88±0.14	2.08±0.14	1.98±0.10
CGK4	2.05±0.04	2.29±0.16	2.17±0.09
CGK5	2.07±0.02	2.31±0.22	2.19±0.11
CS1	2.13±0.23	1.64±0.21	1.88±0.18
CS2	1.81±0.13	1.54±0.36	1.67±0.18
ACS1	1.89±0.15	2.07±0.19	1.98±0.11
ACS2	1.97±0.02	1.97±0.04	1.97±0.02
ACS3	2.06±0.12	2.10±0.14	2.08±0.08
RCS	2.02±0.07	2.03±0.17	2.03±0.08
Simple effect of soil moisture level	1.97±0.03	2.03±0.06	-
P content in the shoot (%)			
M0 (control)	0.15±0.06 ^{aA}	0.12±0.01 ^{bcA}	0.11±0.01 ^a
CM	0.12±0.03 ^{aA}	0.12±0.02 ^{bcA}	0.12±0.02 ^a
CGK1	0.15±0.03 ^{aA}	0.13±0.01 ^{bcA}	0.14±0.01 ^a
CGK2	0.14±0.03 ^{aA}	0.15±0.01 ^{abA}	0.15±0.01 ^a
CGK3	0.15±0.01 ^{aA}	0.17±0.03 ^{aA}	0.16±0.02 ^a
CGK4	0.16±0.02 ^{aA}	0.13±0.01 ^{bcA}	0.15±0.01 ^a
CGK5	0.18±0.01 ^{aA}	0.13±0.01 ^{bcB}	0.16±0.01 ^a
CS1	0.16±0.00 ^{aA}	0.09±0.01 ^{dB}	0.13±0.02 ^a
CS2	0.16±0.02 ^{aA}	0.10±0.00 ^{cdA}	0.13±0.02 ^a
ACS1	0.15±0.01 ^{aA}	0.12±0.00 ^{bcB}	0.14±0.01 ^a
ACS2	0.19±0.00 ^{aA}	0.12±0.01 ^{bcB}	0.15±0.02 ^a
ACS3	0.14±0.04 ^{aA}	0.11±0.02 ^{cdA}	0.12±0.02 ^a
RCS	0.15±0.06 ^{aA}	0.10±0.01 ^{cdA}	0.12±0.03 ^a
Simple effect of soil moisture level	0.15±0.01 ^A	0.12±0.00 ^A	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS).

Values (mean ± SE) within each column followed by different lowercase letters are significantly different ($p < 0.05$) according to Duncan's test, whereas different uppercase letters indicate significant differences ($p < 0.05$) within each row. No statistical significant was detect among means of N content in the shoot.

Table 7 Effect of soil moisture level and mycorrhizal application on shoot N and P uptake of cocoa seedlings at 5 mth after planting

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
N uptake by shoot (mg) *			
M0 (control)	106.51±1.87 ^{aA}	66.94±1.94 ^{aB}	86.72±8.93 ^c
CM	116.59±4.40 ^{aA}	76.90±3.08 ^{aB}	96.75±9.19 ^{bc}
CGK1	121.35±1.73 ^{aA}	89.68±1.46 ^{aB}	105.52±7.15 ^{ab}
CGK2	118.33±5.91 ^{aA}	85.77±10.22 ^{aA}	102.05±8.99 ^{abc}
CGK3	112.61±13.64 ^{aA}	95.24±0.52 ^{aA}	103.92±7.24 ^{abc}
CGK4	123.12±1.17 ^{aA}	91.96±7.39 ^{aB}	107.54±7.73 ^{ab}
CGK5	125.69±7.01 ^{aA}	91.33±5.56 ^{aA}	108.51±8.66 ^{ab}
CS1	123.07±11.51 ^{aA}	69.69±13.44 ^{aB}	96.38±14.32 ^{bc}
CS2	111.93±8.91 ^{aA}	68.18±17.53 ^{aA}	90.06±13.15 ^{bc}
ACS1	129.85±7.97 ^{aA}	84.37±6.10 ^{aB}	107.11±11.12 ^{ab}
ACS2	132.31±3.42 ^{aA}	105.98±6.60 ^{aB}	119.15±6.76 ^a
ACS3	111.10±3.45 ^{aA}	77.22±10.73 ^{aA}	94.16±9.10 ^{bc}
RCS	111.50±1.92 ^{aA}	93.83±11.97 ^{aA}	102.66±6.71 ^{abc}
Simple effect of soil moisture level	118.77±1.99 ^A	84.39±2.76 ^B	-
P uptake by shoot (mg)			
M0 (control)	5.01±0.79 ^{aA}	3.98±0.51 ^{cdA}	4.50±0.48 ^d
CM	7.20±1.61 ^{aA}	5.03±1.11 ^{bcdA}	6.11±1.00 ^{bcd}
CGK1	9.54±1.55 ^{aA}	5.45±0.69 ^{a-dA}	7.49±1.19 ^{abc}

Table 7 Continued

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
CGK2	8.62±1.43 ^{aA}	6.02±1.06 ^{abcA}	7.32±0.99 ^{abc}
CGK3	9.06±0.60 ^{aA}	7.50±1.01 ^{aA}	8.28±0.63 ^{ab}
CGK4	9.86±1.46 ^{aA}	5.43±0.90 ^{a-dB}	7.64±1.25 ^{abc}
CGK5	10.90±0.69 ^{aA}	5.16±0.30 ^{bcdB}	8.03±1.33 ^{abc}
CS1	9.48±0.40 ^{aA}	3.68±0.73 ^{dB}	6.58±1.35 ^{bcd}
CS2	9.89±1.28 ^{aA}	4.22±0.19 ^{cdB}	7.06±1.39 ^{abc}
ACS1	10.32±0.32 ^{aA}	4.92±0.13 ^{bcdB}	7.62±1.22 ^{abc}
ACS2	12.55±0.25 ^{aA}	6.56±0.40 ^{abB}	9.55±1.36 ^a
ACS3	7.07±1.49 ^{aA}	3.79±0.43 ^{cdA}	5.43±1.01 ^{cd}
RCS	8.41±3.53 ^{aA}	4.42±0.40 ^{cdA}	6.41±1.82 ^{bcd}
Simple effect of soil moisture level	9.07±0.45 ^A	5.09±0.24 ^B	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS).

Values (mean ± SE) in each column followed by different lowercase letters are significantly different according to Duncan's test ($p < 0.05$), whereas uppercase letters refer to mean differences within each row.

* = indicator variable to determine the mitigation effect on soil moisture level of 50% FC.

Table 8 Effect of soil moisture level and mycorrhizal application on height and stem diameter of cocoa seedlings at 5 mth after planting.

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhiza types
Seedling height (cm) *			
M0 (control)	27.93±0.74 ^{bcA}	21.30±0.80 ^{cB}	24.62±1.56 ^f
CM	30.63±0.45 ^{abA}	26.33±1.67 ^{abA}	28.48±1.23 ^{a-d}
CGK1	30.92±1.59 ^{abA}	26.42±1.61 ^{abA}	28.67±1.43 ^{a-d}
CGK2	28.00±1.15 ^{bcA}	25.00±0.29 ^{abcA}	26.50±0.85 ^{c-f}
CGK3	26.00±0.58 ^{cA}	24.75±1.52 ^{bcA}	25.38±0.78 ^{ef}
CGK4	27.69±0.47 ^{bcA}	24.50±2.38 ^{bcA}	26.09±1.30 ^{def}
CGK5	29.17±0.08 ^{abcA}	24.75±1.30 ^{bcA}	26.96±1.15 ^{b-f}
CS1	30.42±0.36 ^{abA}	27.33±0.60 ^{abB}	28.88±0.75 ^{abc}
CS2	31.42±2.28 ^{abA}	26.67±1.53 ^{abA}	29.04±1.62 ^{abc}
ACS1	33.08±2.10 ^{aA}	24.33±1.04 ^{bcA}	28.71±2.25 ^{a-d}
ACS2	31.50±0.43 ^{abA}	28.92±0.30 ^{aB}	30.21±0.62 ^a
ACS3	30.67±0.88 ^{abA}	27.83±1.01 ^{abA}	29.25±0.87 ^{ab}
RCS	28.17±0.93 ^{bcA}	27.17±0.44 ^{abA}	27.67±0.51 ^{a-c}
Simple effect of soil moisture level	29.66±0.41 ^A	25.79±0.42 ^B	-
Stem diameter (mm)			
M0 (control)	6.40±0.50 ^{aA}	5.50±0.29 ^{aA}	5.95±0.33 ^a
CM	6.87±0.19 ^{aA}	5.66±0.25 ^{aB}	6.27±0.31 ^a
CGK1	6.92±0.36 ^{aA}	5.96±0.56 ^{aA}	6.44±0.37 ^a
CGK2	7.15±0.33 ^{aA}	5.63±0.16 ^{aB}	6.39±0.38 ^a
CGK3	6.75±0.40 ^{aA}	5.73±0.20 ^{aA}	6.24±0.30 ^a
CGK4	6.57±0.26 ^{aA}	5.72±0.40 ^{aA}	6.15±0.29 ^a
CGK5	6.52±0.29 ^{aA}	5.62±0.14 ^{aA}	6.07±0.25 ^a
CS1	6.46±0.29 ^{aA}	6.04±0.12 ^{aA}	6.25±0.17 ^a
CS2	6.71±0.40 ^{aA}	5.86±0.19 ^{aA}	6.29±0.27 ^a
ACS1	6.90±0.39 ^{aA}	5.71±0.34 ^{aA}	6.31±0.35 ^a
ACS2	7.21±0.12 ^{aA}	6.29±0.15 ^{aA}	6.75±0.22 ^a
ACS3	6.65±0.04 ^{aA}	5.70±0.25 ^{aA}	6.18±0.24 ^a
RCS	6.93±0.27 ^{aA}	6.05±0.28 ^{aA}	6.49±0.26 ^a
Simple effect of soil moisture level	6.77±0.08 ^A	5.80±0.07 ^B	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS).

Values (mean ± SE) within each column followed by different lowercase superscript letters are significantly different according to Duncan's test ($p < 0.05$), whereas different uppercase superscript letters indicate significant differences among means within each row.

* = indicator variable to determine the mitigation effect on soil moisture level of 50% FC.

Table 9 Effect of soil moisture level and mycorrhizal application on number of leaves and leaf area of cocoa seedlings at 5 mth after planting

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
Number of leaves			
M0 (control)	12.67±1.45 ^{aA}	10.00±0.00 ^{aA}	11.33±0.88 ^c
CM	15.27±0.43 ^{aA}	10.50±1.00 ^{aA}	12.88±1.17 ^{abc}
CGK1	14.67±1.17 ^{aA}	11.17±1.20 ^{aA}	12.92±1.08 ^{abc}
CGK2	14.67±0.33 ^{aA}	11.83±0.44 ^{aA}	13.25±0.68 ^{abc}
CGK3	15.00±1.04 ^{aA}	12.17±1.69 ^{aA}	13.58±1.09 ^{ab}
CGK4	12.92±0.22 ^{aA}	9.67±0.67 ^{aB}	11.29±0.79 ^c
CGK5	13.00±0.58 ^{aA}	10.17±1.01 ^{aA}	11.58±0.92 ^{bc}
CS1	14.33±0.17 ^{aA}	10.33±0.67 ^{aB}	12.33±0.95 ^{abc}
CS2	14.17±1.76 ^{aA}	12.33±1.01 ^{aA}	13.25±1.00 ^{abc}
ACS1	14.17±0.60 ^{aA}	13.00±0.58 ^{aA}	13.58±0.45 ^{ab}
ACS2	15.33±0.44 ^{aA}	12.67±0.93 ^{aA}	14.00±0.75 ^a
ACS3	14.67±0.83 ^{aA}	11.00±0.76 ^{aA}	12.83±0.96 ^{abc}
RCS	13.17±0.17 ^{aA}	11.83±1.01 ^{aA}	12.50±0.55 ^{abc}
Simple effect of soil moisture level	14.16±0.24 ^A	11.28±0.27 ^B	-
Leaf area (cm ²)			
M0 (control)	81.29±7.63 ^{aA}	80.87±9.21 ^{aA}	81.08±5.35 ^a
CM	97.08±8.22 ^{aA}	93.90±7.39 ^{aA}	95.49±4.99 ^a
CGK1	108.67±7.04 ^{aA}	80.24±3.68 ^{aA}	94.46±7.28 ^a
CGK2	97.80±7.08 ^{aA}	76.62±8.69 ^{aA}	87.21±6.89 ^a
CGK3	84.26±0.52 ^{aA}	87.72±10.21 ^{aA}	85.99±4.64 ^a
CGK4	104.93±1.53 ^{aA}	80.23±6.41 ^{aA}	92.58±6.26 ^a
CGK5	97.96±5.66 ^{aA}	82.58±6.23 ^{aA}	90.27±5.10 ^a
CS1	98.50±17.30 ^{aA}	81.44±3.24 ^{aA}	89.97±8.75 ^a
CS2	108.85±2.30 ^{aA}	87.66±5.04 ^{aA}	98.25±5.35 ^a
ACS1	116.56±15.70 ^{aA}	80.08±4.26 ^{aA}	98.32±10.93 ^a
ACS2	109.32±3.71 ^{aA}	87.36±2.19 ^{aB}	98.34±5.27 ^a
ACS3	99.17±5.27 ^{aA}	65.19±1.84 ^{aB}	82.18±8.00 ^a
RCS	96.59±5.80 ^{aA}	95.97±8.17 ^{aA}	96.28±4.48 ^a
Simple effect of soil moisture level	100.07±2.44 ^A	83.07±1.92 ^A	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS).

Values (mean ± SE) within each column followed by different lowercase superscript letters are significantly different according to Duncan's test ($p < 0.05$), whereas different uppercase superscript letters indicate significant differences among means within each row.

Table 10 Effect of soil moisture level and mycorrhizal application on root volume and length of cocoa seedlings at 5 mth after planting

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhiza types
Root volume (m ³)			
M0 (control)	13.33±1.67 ^{aA}	9.33±0.93 ^{aA}	11.33±1.24 ^b
CM	15.67±3.32 ^{aA}	12.50±1.44 ^{aA}	14.08±1.77 ^{ab}
CGK1	14.50±1.32 ^{aA}	10.67±0.44 ^{aB}	12.58±1.06 ^b
CGK2	14.83±1.45 ^{aA}	8.67±1.01 ^{aA}	11.75±1.59 ^b
CGK3	14.17±0.88 ^{aA}	11.33±2.68 ^{aA}	12.75±1.41 ^b
CGK4	14.80±2.60 ^{aA}	9.67±0.33 ^{aA}	12.23±1.64 ^b
CGK5	14.10±0.90 ^{aA}	10.10±0.74 ^{aA}	12.10±1.03 ^b
CS1	14.67±0.17 ^{aA}	11.83±1.48 ^{aA}	13.25±0.92 ^b
CS2	15.00±0.00 ^{aA}	11.67±1.36 ^{aA}	13.33±0.96 ^b
ACS1	14.83±1.30 ^{aA}	10.50±1.04 ^{aB}	12.67±0.22 ^b
ACS2	16.43±1.29 ^{aA}	15.50±1.50 ^{aA}	15.97±0.91 ^a
ACS3	13.33±1.67 ^{aA}	10.60±1.14 ^{aA}	11.97±1.09 ^b
RCS	12.17±0.17 ^{aA}	11.17±0.60 ^{aA}	11.67±0.36 ^b
Simple effect of soil moisture level	14.45±0.40 ^A	11.04±0.40 ^A	-
Root length (cm)			
M0 (control)	40.33±5.49 ^{aA}	34.67±1.48 ^{aA}	37.50±2.94 ^a
CM	46.50±3.97 ^{aA}	38.53±0.62 ^{aA}	42.52±2.53 ^a
CGK1	47.43±6.37 ^{aA}	34.77±1.67 ^{aA}	41.10±4.08 ^a
CGK2	50.33±2.80 ^{aA}	36.60±5.02 ^{aA}	43.47±4.01 ^a

Table 10 Continued

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhiza types
CGK3	50.67±3.42 ^{aA}	35.77±3.83 ^{aB}	43.22±4.05 ^a
CGK4	42.37±1.73 ^{aA}	33.27±3.02 ^{aA}	37.82±2.56 ^a
CGK5	42.20±0.38 ^{aA}	40.87±3.68 ^{aA}	41.53±1.68 ^a
CS1	50.77±5.68 ^{aA}	33.00±1.89 ^{aB}	41.88±4.79 ^a
CS2	44.60±4.23 ^{aA}	35.83±0.73 ^{aA}	40.22±2.74 ^a
ACS1	45.93±1.16 ^{aA}	30.77±2.80 ^{aA}	38.35±3.65 ^a
ACS2	45.17±2.13 ^{aA}	34.10±4.27 ^{aA}	39.63±3.27 ^a
ACS3	44.33±2.24 ^{aA}	30.10±4.27 ^{aB}	37.22±3.34 ^a
RCS	44.77±2.80 ^{aA}	33.17±0.44 ^{aB}	38.97±2.89 ^a
Simple effect of soil moisture level	45.80±0.99 ^A	34.73±0.78 ^B	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM =commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS).

Values (mean ± SE) within each column followed by different lowercase superscript letters are significantly different according to Duncan's test ($p < 0.05$), whereas different uppercase superscript letters indicate significant differences among means within each row.

Table 11 Effect of soil moisture level and mycorrhizal application on shoot fresh and dry weight of cocoa seedlings at 5 mth after planting

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
Shoot fresh weight (g) *			
M0 (control)	11.94±0.56 ^{aA}	7.55±0.35 ^{aB}	9.74±1.03 ^a
CM	13.01±0.44 ^{aA}	9.54±0.58 ^{aB}	11.28±0.84 ^a
CGK1	14.25±2.10 ^{aA}	8.69±0.49 ^{aA}	11.47±1.57 ^a
CGK2	13.65±1.17 ^{aA}	9.39±1.56 ^{aA}	11.52±1.29 ^a
CGK3	12.96±0.37 ^{aA}	10.77±0.96 ^{aA}	11.87±0.67 ^a
CGK4	12.74±1.24 ^{aA}	9.04±0.54 ^{aA}	10.89±1.02 ^a
CGK5	13.26±0.58 ^{aA}	8.37±0.42 ^{aB}	10.81±1.14 ^a
CS1	12.65±0.70 ^{aA}	8.63±0.98 ^{aA}	10.64±1.05 ^a
CS2	14.26±1.15 ^{aA}	9.34±0.23 ^{aB}	11.80±1.22 ^a
ACS1	15.13±1.59 ^{aA}	9.55±0.40 ^{aB}	12.34±1.45 ^a
ACS2	13.13±0.31 ^{aA}	12.12±1.79 ^{aA}	12.63±0.84 ^a
ACS3	11.20±0.20 ^{aA}	8.62±0.82 ^{aA}	9.91±0.69 ^a
RCS	12.12±0.26 ^{aA}	10.84±0.78 ^{aA}	11.48±0.46 ^a
Simple effect of soil moisture level	13.10±0.28 ^A	9.42±0.28 ^B	-
Shoot dry weight (g) *			
M0 (control)	5.36±0.05 ^{aA}	3.30±0.21 ^{aB}	4.33±0.47 ^d
CM	5.99±0.02 ^{aA}	4.15±0.31 ^{aB}	5.07±0.43 ^{bcd}
CGK1	6.52±0.45 ^{aA}	4.08±0.36 ^{aB}	5.30±0.60 ^{bc}
CGK2	6.20±0.17 ^{aA}	4.00±0.68 ^{aA}	5.10±0.58 ^{bc}
CGK3	5.93±0.30 ^{aA}	4.61±0.27 ^{aA}	5.27±0.35 ^{bc}
CGK4	6.01±0.11 ^{aA}	4.01±0.28 ^{aB}	5.02±0.46 ^{bcd}
CGK5	6.07±0.28 ^{aA}	3.99±0.29 ^{aB}	5.03±0.50 ^{bcd}
CS1	5.81±0.16 ^{aA}	4.19±0.28 ^{aB}	5.00±0.39 ^{bcd}
CS2	6.26±0.60 ^{aA}	4.37±0.13 ^{aA}	5.31±0.50 ^{bc}
ACS1	6.89±0.13 ^{aA}	4.10±0.11 ^{aB}	5.50±0.63 ^{ab}
ACS2	6.73±0.18 ^{aA}	5.40±0.39 ^{aA}	6.06±0.35 ^a
ACS3	5.44±0.45 ^{aA}	3.66±0.32 ^{aA}	4.55±0.47 ^{cd}
RCS	5.53±0.10 ^{aA}	4.63±0.54 ^{aA}	5.08±0.32 ^{bcd}
Simple effect of soil moisture level	6.06±0.10 ^A	4.19±0.11 ^B	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM =commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS).

Values (mean ± SE) within each column followed by different lowercase superscript letters are significantly different according to Duncan's test ($p < 0.05$), whereas different uppercase superscript letters indicate significant differences among means within each row.

* = indicator variable to determine the mitigation effect on soil moisture level of 50% FC.

Table 12 Effect of soil moisture level and mycorrhizal application on root fresh and dry weight of cocoa seedlings at 5 mth after planting

Type of mycorrhiza	Soil moisture level of 100% FC	Soil moisture level of 50% FC	Simple effect of mycorrhizal types
Root fresh weight (g)			
M0 (control)	11.81±1.55	8.75±0.56	10.28±1.00
CM	13.09±1.81	12.48±0.83	12.78±0.90
CGK1	12.49±0.96	9.17±1.81	10.83±1.18
CGK2	12.17±1.19	9.34±1.31	10.76±1.01
CGK3	12.79±0.76	10.10±0.85	11.44±0.79
CGK4	12.78±3.11	9.16±0.81	10.97±1.65
CGK5	13.25±0.12	10.42±0.84	11.83±0.74
CS1	12.34±0.80	11.13±1.36	11.74±0.75
CS2	13.41±1.05	8.89±1.40	11.15±1.28
ACS1	13.08±2.11	10.41±0.71	11.74±1.16
ACS2	13.49±1.51	13.64±1.91	13.56±1.09
ACS3	11.56±1.48	9.22±0.73	10.39±0.91
RCS	10.73±1.03	10.61±0.69	10.67±0.55
Simple effect of soil moisture level	12.54±0.37	10.25±0.35	-
Root dry weight (g)			
M0 (control)	3.45±0.33 ^{aA}	2.34±0.18 ^{aA}	2.90±0.30 ^b
CM	4.15±0.18 ^{aA}	3.49±0.40 ^{aA}	3.82±0.24 ^a
CGK1	3.71±0.32 ^{aA}	2.99±0.25 ^{aA}	3.35±0.24 ^{ab}
CGK2	3.72±0.33 ^{aA}	2.32±0.27 ^{aB}	3.02±0.37 ^b
CGK3	3.77±0.27 ^{aA}	2.71±0.16 ^{aA}	3.24±0.27 ^{ab}
CGK4	3.60±0.45 ^{aA}	2.52±0.07 ^{aA}	3.06±0.32 ^b
CGK5	3.68±0.42 ^{aA}	2.76±0.39 ^{aA}	3.22±0.33 ^{ab}
CS1	4.10±0.21 ^{aA}	2.92±0.29 ^{aA}	3.51±0.31 ^{ab}
CS2	3.66±0.27 ^{aA}	2.56±0.19 ^{aA}	3.11±0.29 ^b
ACS1	3.64±0.45 ^{aA}	2.74±0.39 ^{aA}	3.19±0.33 ^{ab}
ACS2	4.16±0.22 ^{aA}	3.55±0.48 ^{aA}	3.85±0.27 ^a
ACS3	3.57±0.47 ^{aA}	2.47±0.11 ^{aA}	3.02±0.33 ^b
RCS	3.09±0.27 ^{aA}	2.79±0.24 ^{aA}	2.94±0.17 ^b
Simple effect of soil moisture level	3.72±0.09 ^A	2.78±0.09 ^B	-

FC = field capacity; M0 = control, with no added mycorrhizae; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, the Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS).

Values (mean ± SE) within each column followed by different lowercase superscript letters are significantly different according to Duncan's test ($p < 0.05$), whereas different uppercase superscript letters indicate significant differences among means within each row. No significant differences were observed among mean values for Root fresh weight ($p \geq 0.05$).

Ayegboyin and Akinrinde (2016) indicated that water stress of 50% FC with watering intervals of 5 d and 7 d in several cocoa cultivars could reduce seedling height by 20–30%. Other studies revealed that water stress conditions led to a decrease in the height of cocoa seedlings (Chibuike and Daymond, 2015; Alban et al., 2016; Djan et al., 2018; Zakariyya and Indradewa, 2018), while the application of mycorrhiza significantly increased the height of cocoa seedlings by 31% compared to the control (Rokhmah et al., 2020). Furthermore, the height of cocoa seedlings with AMF inoculation was 125% higher than the control (Kaba et al., 2021). Notably, the application of mycorrhiza to cocoa seeds also increased plant height (Iglesias et al., 2011; Tchameni et al., 2012). The reduction in cocoa plant growth due to water stress is affected by various factors, including the plant's developmental stage, the severity of the

drought stress and the specific cocoa clones involved (Alban et al., 2016; Ayegboyin and Akinrinde, 2016; Kaba et al., 2021). The plant response to water stress was to reduce photosynthetic activity and metabolic processes, resulting in a decrease in several plant growth variables. In addition, a water deficit leads to a reduction in leaf cell size, resulting in lower photosynthetic activity (Ayegboyin and Akinrinde, 2016; Zakariyya and Indradewa, 2018). At the same time, the rate of photosynthesis decreases due to stomatal closure, which limits the movement of CO₂ through tissues in both the stomata and mesophyll (Kapoor et al., 2020). However, excessive stomatal closure causes degradation of photosynthetic pigments (Ayegboyin and Akinrinde, 2016). Additionally, in water deficit, the activity of enzymes, such as nitrate reductase, can decrease (Mandi et al., 2018).

On the other hand, Alban et al. (2016) demonstrated that the cocoa crown decreased due to a soil moisture level of 50% FC in several hybrid cocoa clones. However, arbuscular mycorrhizal inoculation at 6 g/tree increased the dry weight of cocoa shoots (Nasaruddin and Ridwan, 2018). In this regard, water limitation physiologically inhibits the activity of nitrate reductase, an enzyme essential for protein production, the regulation of photosynthesis, water use efficiency and nitrogen assimilation in cocoa plants, with the inhibition impacting overall plant health and productivity (Nguyen et al., 2014; Mandi et al., 2018; Zakariyya and Indradewa, 2018). A low growth rate of the cocoa plant indicates limited photosynthesis, due to the breakdown of photosynthetic pigments, reduced CO₂ absorption and a low water content (Zakariyya and Indradewa, 2018).

Mitigation effect of mycorrhizae on soil moisture level at 50% field capacity

The previous analysis of simple effects and specific effects revealed significant differences (Table 1 and Table 4-12). The simple effect analysis demonstrated significant differences between SML at 100% and 50% FC in 10 variables: root infection, N uptake by the shoot, P uptake by the shoot, seedling height, stem diameter, number of leaves, root length,

shoot fresh weight, shoot dry weight and root dry weight (Table 1, Table 5 and Tables 7–12, respectively). However, the analysis of specific effects, particularly in the M0 treatment, identified only four variables: N uptake by the shoot, seedling height, shoot fresh weight and shoot dry weight (Table 7, Table 8 and Table 11). These findings indicated that analyzing both factors (MA and SML) simultaneously could obscure their specific effects. Therefore, the current study focused on examining 100% FC compared to 50% FC for each MA treatment to address the research hypothesis.

Based on this analysis, four variables—N uptake, seedling height, shoot fresh weight and shoot dry weight—were used as indicators to determine the mycorrhizal types that could mitigate water limitations at 50% FC, due to the SML 100% FC in M0 being significantly better than the 50% FC, as illustrated in Tables 7, 8 and 11. However, three mycorrhizal types, CGK3, ACS3 and RCS, consistently demonstrated similar performance at 100% and 50% FC for all indicator variables. In contrast, the other mycorrhizal types only occurred in a maximum of three indicator variables (Fig. 1). Therefore, these three mycorrhizal types were deemed capable of overcoming water limitation at 50% FC. Fig. 2 illustrates the comparison between 100% and 50% FC for the three selected mycorrhizal types and the control based on the four indicator variables.

Type of mycorrhiza	Variable observed															
	1	2	3	4	5*	6	7*	8	9	10	11	12	13*	14*	15	16
M0 (control)																
CM																
CGK1																
CGK2																
CGK3																
CGK4																
CGK5																
CS1																
CS2																
ACS1																
ACS2																
ACS3																
RCS																

Fig. 1 Mitigation effect of 12 mycorrhizal types and the control under 50% field capacity (FC). Gray-shaded cells indicate a mitigation effect under 50% FC, where performance at 50% FC was equal to or greater than that at 100% FC. Blue-shaded cells indicate mycorrhizal types selected for tolerance to 50% FC. FC = field capacity; M0 = control without mycorrhizal application; CM = commercial mycorrhizae from Badan Pengkajian dan Penerapan Teknologi, Agency for the Assessment and Application Technology (BPPT); CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; CS = cocoa sourced from Sukabumi City in West Java Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City; RCS = robusta coffee sourced from Sukabumi City (RCS); * = indicator variable to determine the mitigation effect on soil moisture level of 50% FC (M0 in 100% FC > 50% FC); blue shaded cells indicate mycorrhizal types selected to overcome 50% FC; (1) = available P in soil, (2) = root infection; (3) = N content in shoot; (4) = P content in shoot; (5) = N uptake by shoot; (6) = P uptake by shoot; (7) = seedling height; (8) = stem diameter; (9) = number of leaves; (10) = leaf area; (11) = root volume; (12) = root length; (13) = shoot fresh weight; (14) = shoot dry weight; (15) = root fresh weight; (16) = root dry weight

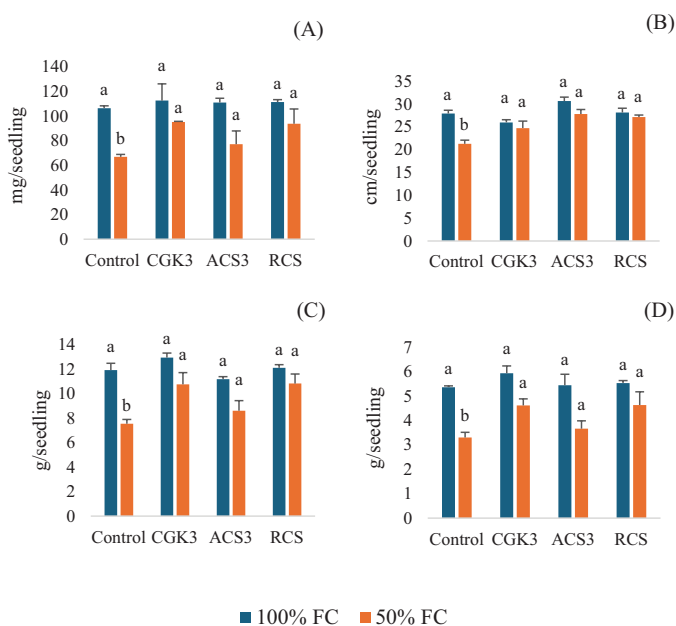


Fig. 2 Comparison between soil moisture levels of 100% and 50% field capacity (FC) for three selected mycorrhizal types and control on N uptake by: (A) shoot; (B) seedling height; (C) shoot fresh weight; (D) shoot dry weight, where CGK = cocoa sourced from Gunung Kidul Regency in Yogyakarta Province, Indonesia; ACS = Arabica coffee sourced from Sukabumi City in West Java Province, Indonesia; RCS = robusta coffee sourced from Sukabumi City. Bars represent mean values and error bars represent SE. Bars with different lowercase letters are significantly different according to Duncan's test ($p < 0.05$).

Based on the current results, mycorrhizae from the rhizosphere of cocoa, Arabica coffee and robusta coffee plants collected from various locations enhanced the N uptake and promoted the growth of cocoa seedlings. In general, the mycorrhizal types CGK3, ACS3 and RCS were relatively better at overcoming water limitations at 50% FC. The effectiveness of mycorrhizal type was significantly affected by interactions among the soil, host plants and various environmental factors. Plant responses to mycorrhiza have been reported to depend on the experimental conditions, nutrient availability and the specific characteristics of the plants (Martin and van der Heijden, 2024). Furthermore, interactions between inoculated mycorrhizae and indigenous mycorrhizae in the soil can influence inoculation success and these interactions can be synergistic or antagonistic. In the current study, the consortium formed by *Scutellospora* sp. and *Glomus* sp. in strain CGK3 may have been compatible with the native mycorrhizae present in the soil, leading to increased activity in overcoming water limitations at 50% FC. This would be consistent with Berruti et al. (2016), who stated incompatibility between

inoculated mycorrhizae and indigenous mycorrhizae in non-sterile soil could reduce inoculation success. In this regard, to enhance inoculation success, future studies should prioritize the identification and screening of mycorrhizal types that are phylogenetically more compatible (Basiru and Hijri, 2022). Notably, while ACS3 and RCS belong to the same genus, they originate from different plant sources (cocoa and coffee, respectively). Specific interactions between the source plant and the mycorrhiza may influence their performance. Additionally, the acidic soil with a pH of 4.11 in the current study (Table 1) may have affected mycorrhizal activity. The mycorrhizal types examined in the current study may have varying adaptability to acidic soil conditions, which would affect their ability to cope with water limitations at 50% FC. This aligned with the results of Alkobaisy (2022), who studied the response of various mycorrhizal types to pH. Furthermore, one possible reason that CGK3, ACS3 and RCS outperformed the other types in the current study is their differing phytohormone contents. Pons et al. (2020) revealed the presence of phytohormones in mycorrhizae, which could influence their performance.

Conclusion

This study highlighted the effectiveness of mycorrhizal fungi in overcoming soil water limitations at 50% field capacity. Based on the findings, a soil moisture level of 50% field capacity decreased nitrogen uptake by the shoot, as well as the height, shoot fresh weight and shoot dry weight of cocoa seedlings; however, it did not induce drought stress. Three mycorrhizal types were identified—CGK3, ACS3 and RCS—that effectively managed water limitations at 50% field capacity. These findings should expand the diversity of superior mycorrhizal types in overcoming soil water limitations for cocoa seedlings. Future studies should focus on identifying and screening indigenous mycorrhizae in non-sterile soils to assess their compatibility with inoculated types, as well as the phytohormone content of each mycorrhizal strain.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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