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STAGE-SPECIFIC COCOA SEEDLING RESPONSES TO MYCORRHIZA AND NPK FERTILIZATION

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ABSTRACT

Cocoa seedling quality depends on efficient nursery nutrition, but the benefits of biological and mineral inputs may vary across early growth stages. This study evaluated stage-specific growth responses of cocoa seedlings (Theobroma cacao L.) to mycorrhiza and NPK fertilizer. A 3 x 3 factorial randomized block design with three replications was used, combining mycorrhiza at 0, 40, and 50 g/polybag with NPK 16:16:16 at 4, 6, and 10 g/polybag. Plant height, leaf number, leaf length, and stem diameter were measured at 2-12 weeks after planting (WAP), while root volume was assessed at 12 WAP. Analysis of variance was followed by Duncan's Multiple Range Test at 5% when significant. Mycorrhiza did not significantly affect any measured trait, and no mycorrhiza x NPK interaction was detected. In contrast, NPK significantly affected leaf number from 4 to 12 WAP and leaf length at 4, 6, and 8 WAP. At 12 WAP, 10 g NPK produced 9.96 leaves, while at 8 WAP it produced the greatest leaf length (6.93 cm); plant height, stem diameter, and root volume remained statistically unchanged. These results reveal a stage- and organ-specific nutrient response: NPK first enhanced leaf development without producing parallel short-term gains in structural or root traits. Under the tested nursery conditions, increasing mineral nutrition was more immediately effective than mycorrhizal inoculation, while longer-term studies should verify root colonization and nutrient status.

Keywords: Theobroma cacao; arbuscular mycorrhiza; NPK fertilizer; seedling growth; nutrient interaction.

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) remains an economically important plantation crop in Indonesia, and nursery quality is a strategic starting point for sustaining field establishment and productivity. Recent national estate-crop statistics continue to document cocoa as a major permanent crop, while sector analyses emphasize the need to improve planting materials and cultivation practices as part of broader efforts to strengthen production and quality (BPS-Statistics Indonesia, 2025; Ariningsih et al., 2021). A vigorous nursery seedling should develop a balanced canopy, stem, and root system before transplanting. Nutrient management during this period is therefore not only a matter of maximizing size, but also of matching nutrient supply to the physiological stage at which particular organs are most responsive.

Arbuscular mycorrhizal fungi (AMF) are widely considered biological partners that can extend the effective absorptive area of roots, improve access to relatively immobile nutrients such as phosphorus, and influence plant water and nutrient relations (Begum et al., 2019; Ferrol et al., 2019). However, growth responses are not guaranteed merely by inoculation. The functional outcome depends on host-fungus compatibility, colonization intensity, substrate fertility, phosphorus availability, environmental conditions, and the carbon cost of maintaining the symbiosis (Smith & Smith, 2012; Treseder, 2013). A recent meta-analysis confirms that AMF inoculation generally improves plant biomass and nitrogen and phosphorus nutrition, but also shows substantial variation among experimental contexts (Wu et al., 2024). Reviews in horticultural crops likewise emphasize that AMF benefits are strongly conditioned by crop and growing environment (Zhu et al., 2022).

Mineral NPK fertilization supplies nitrogen, phosphorus, and potassium that support chlorophyll formation, energy transfer, cell division, osmotic regulation, and enzyme activity. Cocoa nursery studies show that NPK responses vary with dose, genotype, media, and accompanying amendments. NPK has improved vegetative traits in cocoa seedlings in several experiments (Junenti et al., 2023; Harahap & Maulana, 2024), whereas other factorial studies have reported selective main effects or weak interactions depending on the substrate and co-applied organic input (Sari & Alfrizon, 2023; Hartawan et al., 2024). More recent work also demonstrates that 10 g NPK can contribute strongly to cocoa seedling vigor when combined with a growth regulator, illustrating the importance of treatment context (Kusmiati et al., 2026).

The combined use of biological and mineral inputs is attractive because AMF could, in principle, increase nutrient-use efficiency while NPK supplies immediately available macronutrients. Yet evidence from cocoa and other nursery systems is mixed. Positive interactions have been observed when NPK is combined with cocoa-pod compost, Asystasia compost, or oil-palm empty-fruit-bunch bokashi (Depari et al., 2018; Prasatyio et al., 2025; Hutauruk & Zahrah, 2026). Biofertilizer studies in cacao also show that biological amendments can influence seedling growth and soil microbial populations (Victoria & Aggangan, 2023). At the same time, classic nursery research demonstrated that cacao can respond strongly to vesicular-arbuscular mycorrhiza under favorable inoculation conditions (Cuenca et al., 1990), implying that a lack of response in another environment should be interpreted as context dependent rather than as evidence that mycorrhiza is intrinsically ineffective.

An additional issue is temporal resolution. Reporting only the final seedling size can conceal the possibility that treatments act first on rapidly developing organs such as leaves and only later on stem or root structure. Recent crop experiments published in Algebra also illustrate that factorial treatments may influence specific traits at specific weeks after planting rather than producing uniform effects across all observations (Hasibuan et al., 2024; Revina Azhari et al., 2025). Accordingly, the present study reframes the original nursery experiment around a stage-specific question: do mycorrhiza and NPK fertilizer affect cocoa seedling organs in the same way and at the same time during the first 12 weeks? The study aimed to quantify the main and interactive effects of mycorrhiza and NPK on plant height, leaf number, leaf length, stem diameter, and root volume, with particular attention to when significant responses emerged. The working hypothesis was that mycorrhiza and NPK, individually and in combination, would improve early cocoa seedling growth; the stage-specific analysis was used to identify which components of that hypothesis were supported.

RESEARCH METHODS

Study site and experimental design

The experiment was conducted from June to September 2025 at Jalan Setia Makmur, Gang Pertama, Sunggal Kanan, Deli Serdang, North Sumatra, Indonesia. The study used a factorial randomized block design with two treatment factors and three replications. The first factor was mycorrhiza (M): M0 = 0 g/polybag, M1 = 40 g/polybag, and M2 = 50 g/polybag. The second factor

was NPK Mutiara 16:16:16 (N): N1 = 4 g/polybag, N2 = 6 g/polybag, and N3 = 10 g/polybag. The factorial arrangement produced nine treatment combinations (M0N1 to M2N3) and 27 experimental plots.

Each plot contained four cocoa plants, giving 108 plants in the experiment. Three plants per plot were designated as samples, resulting in 81 sampled plants. The stated experimental layout used 80 cm between replications, 30 cm between plots, and 45 x 45 cm plant spacing. The materials included cocoa planting material, mycorrhiza, NPK 16:16:16 fertilizer, topsoil, and sand. Topsoil was placed in 3-kg polybags, and the assigned mycorrhiza dose was incorporated into the planting medium according to treatment.

Crop establishment and maintenance

The experimental area was cleared of weeds and plant residues before the polybags were arranged by treatment. Cocoa was planted in the prepared medium and watered to establish contact between planting material and substrate. Irrigation was applied in the morning (07:00-09:00) and afternoon (16:00-18:00), with watering reduced or omitted when rainfall maintained adequate substrate moisture. Abnormal or dead plants were replaced at 2 WAP using planting material of comparable age and treatment status. Weeding began at 1 WAP and was performed manually to limit competition within and around the polybags.

Growth measurements

Plant height, leaf number, leaf length, and stem diameter were measured at 2, 4, 6, 8, 10, and 12 WAP. Plant height was measured from the stem base to the highest growing point. Leaf number included fully expanded leaves. Leaf length was measured from the leaf base to the tip. Stem diameter was measured with a caliper. Root volume was measured at the end of the experiment (12 WAP) by water displacement after roots had been cleaned; the increase in water volume after root immersion was recorded as root volume.

Statistical analysis

Data were analyzed using analysis of variance for the factorial randomized block design. The statistical model was Yijk = mu + rho_i + alpha_j + beta_k + (alpha beta)_jk + epsilon_ijk, where mu is the overall mean, rho_i is the block effect, alpha_j is the mycorrhiza effect, beta_k is the NPK effect, (alpha beta)_jk is the interaction effect, and epsilon_ijk is the experimental error. When the F-test indicated a significant treatment effect, mean separation was conducted using Duncan's Multiple Range Test (DMRT) at the 5% significance level. Because the source dataset reports significance decisions and DMRT group letters rather than exact P values, the results below retain the reported alpha = 0.05 inference without inventing unreported probabilities.

RESULTS AND DISCUSSION

Overall growth response at 12 WAP

The response pattern at 12 WAP was highly trait dependent. Mycorrhiza did not significantly affect plant height, leaf number, leaf length, stem diameter, or root volume. NPK likewise did not significantly alter final plant height, leaf length, stem diameter, or root volume at 12 WAP, although it significantly affected leaf number. The mycorrhiza x NPK interaction was non-significant for all five traits. Thus, the most consistent statistical signal in the experiment was not a generalized increase in whole-seedling size, but a selective response of leaf development to mineral fertilization.

Table 1. Main treatment means for cocoa seedling growth at 12 WAP

Treatment	Plant height (cm)	Leaf number	Leaf length (cm)	Stem diameter (mm)	Root volume (mL)
M0 (0 g mycorrhiza)	18.02	9.19	11.94	6.48	5.62
M1 (40 g mycorrhiza)	18.33	9.63	12.07	6.29	6.13
M2 (50 g mycorrhiza)	18.08	9.26	12.17	6.11	5.89
M effect	ns	ns	ns	ns	ns
N1 (4 g NPK)	17.86	8.43 a	11.72	6.14	5.80
N2 (6 g NPK)	18.30	9.67 b	12.23	6.50	5.87
N3 (10 g NPK)	18.26	9.96 b	12.21	6.22	5.96
N effect	ns	*	ns	ns	ns
M x N effect	ns	ns	ns	ns	ns

*Note. * = significant at the 5% level; ns = not significant. Within the NPK leaf-number means, values followed by different letters differ according to DMRT at 5%. Root volume was measured at 12 WAP only.*

Among mycorrhiza levels, M1 (40 g/polybag) had the largest descriptive means for height (18.33 cm), leaf number (9.63), and root volume (6.13 mL), but none differed statistically from M0 or M2. Among NPK levels, N3 (10 g/polybag) produced the greatest leaf number (9.96), while N2 and N3

belonged to the same DMRT group and both exceeded N1 at 12 WAP. Final height differed by only 0.44 cm among NPK means, and final root volume differed by only 0.16 mL, which is consistent with the non-significant ANOVA results for these traits.

Stage-specific leaf response to NPK

The temporal data clarified why leaf number was the clearest NPK-responsive trait. At 2 WAP, the three NPK treatments were statistically similar. From 4 WAP onward, N2 and N3 consistently formed a higher DMRT group than N1, and the separation persisted through 12 WAP. This pattern suggests that the mineral nutrient effect emerged after initial establishment and was maintained during active vegetative development rather than being present immediately after planting.

Table 2. NPK effects on leaf number across 2-12 WAP

NPK dose	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
N1 (4 g)	2.36	4.42 a	5.51 a	6.38 a	7.34 a	8.43 a
N2 (6 g)	2.37	4.87 b	6.13 b	7.25 b	8.44 b	9.67 b
N3 (10 g)	2.53	5.08 b	6.30 b	7.44 b	8.58 b	9.96 b
NPK effect	ns	*	*	*	*	*

*Note. Within each WAP column, means followed by different letters differ according to DMRT at 5%; * = significant; ns = not significant.*

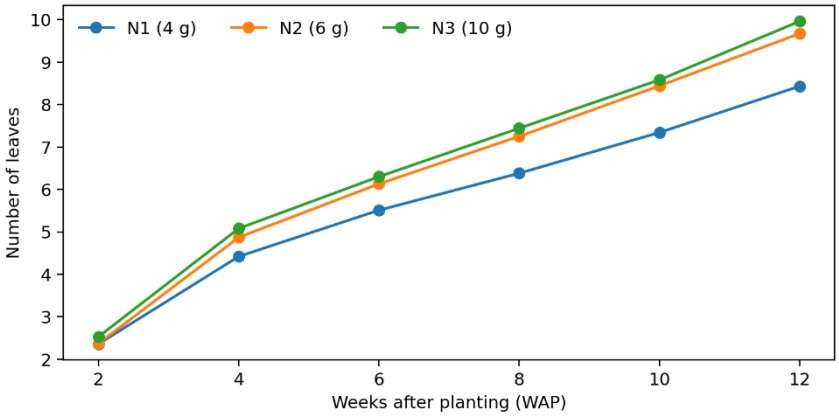


Figure 1. Stage-specific leaf-number response of cocoa seedlings to NPK dose

Leaf length showed a narrower response window. NPK significantly affected leaf length at 4, 6, and 8 WAP, but not at 2, 10, or 12 WAP. N3 produced the greatest mean leaf length during the significant window: 2.63 cm at 4 WAP, 4.33 cm at 6 WAP, and 6.93 cm at 8 WAP. By 10-12 WAP, numerical differences had converged and were no longer statistically distinguishable. The loss of significance at later observations is important because it shows that a treatment can accelerate an organ's early expansion without necessarily creating a permanent difference in final size.

Table 3. NPK effects on leaf length across 2-12 WAP

NPK dose	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
N1 (4 g)	1.28	2.51 a	3.62 a	5.62 a	8.22	11.72
N2 (6 g)	1.25	2.51 a	3.80 a	6.10 a	8.73	12.23
N3 (10 g)	1.31	2.63 b	4.33 b	6.93 b	8.71	12.21
NPK effect	ns	*	*	*	ns	ns

*Note. Within each WAP column, means followed by different letters differ according to DMRT at 5%; * = significant; ns = not significant.*

A stage-specific leaf response is physiologically plausible because nitrogen is closely associated with chlorophyll and protein formation, phosphorus supports energy transfer and cellular metabolism, and potassium contributes to osmotic and enzymatic regulation. In a young seedling, these functions can appear first as faster initiation and expansion of leaves before measurable differences accumulate in stem diameter, height, or root volume. Comparable cocoa nursery studies have reported vegetative responses to NPK, but the magnitude and affected traits differ with fertilizer rate, genotype, substrate, and co-treatment (Junenti et al., 2023; Harahap & Maulana, 2024; Sari & Alfrizon, 2023). Hartawan et al. (2024) found strong growth gains when NPK was integrated with liquid organic fertilizer, whereas Kusmiati et al. (2026) reported broad improvements when NPK was combined with nitrophenol compounds. These contrasts support interpreting the present response as conditional rather than as a universal dose rule.

The selective response also aligns with the broader observation that factorial crop treatments can produce temporally specific effects. Hasibuan et al. (2024) reported treatment effects that became evident at particular weeks in beet, and Revina Azhari et al. (2025) similarly observed time-dependent

vegetative responses in cherry tomato. For cocoa nursery management, the practical implication is that leaf traits may function as earlier indicators of nutritional response than stem or root traits during the first three months. However, leaf acceleration alone should not be equated with improved transplant quality unless subsequent structural growth and biomass confirm that advantage.

Why mycorrhiza did not produce a significant short-term growth response

The absence of a significant mycorrhiza main effect contrasts with studies where cacao seedlings responded positively to effective mycorrhizal inoculation. Cuenca et al. (1990), for example, documented increased cacao seedling growth and nutrient uptake under nursery conditions when roots were successfully associated with vesicular-arbuscular mycorrhiza. Victoria and Aggangan (2023) also showed that biological fertilizer regimes can improve cacao growth and alter soil microbial populations. General AMF literature likewise demonstrates well-established mechanisms for increasing nutrient acquisition and stress tolerance (Begum et al., 2019; Zhu et al., 2022), and meta-analytic evidence indicates an overall positive effect of AMF inoculation on biomass and N-P nutrition (Wu et al., 2024).

Nevertheless, AMF function is not equivalent to inoculum dose. The present experiment did not quantify root colonization percentage, hyphal development, or plant tissue phosphorus, so it cannot determine whether the non-significant growth response resulted from weak colonization, adequate native nutrient supply, a short establishment period, or another biological constraint. This distinction matters because root colonization itself does not always translate proportionally into growth; the benefit depends on the balance between fungal carbon demand and nutrient delivery to the host (Smith & Smith, 2012; Treseder, 2013). Accordingly, the current data support the narrower statement that 40-50 g of the tested mycorrhizal input did not produce a detectable growth advantage during 12 WAP under the tested topsoil nursery conditions.

Mycorrhiza x NPK interaction and nutrient context

No mycorrhiza x NPK interaction was significant for height, leaf number, leaf length, stem diameter, or root volume. This result means that the descriptive maxima of individual treatment combinations should not be interpreted as statistically superior combinations. At 12 WAP, the highest numerical means were M1N3 for height (18.64 cm) and leaf number (10.36), M2N3 for leaf length (12.50 cm), M0N2 for stem diameter (6.95 mm), and M1N1 for root volume (6.41 mL). These maxima occurred under different combinations, reinforcing the absence of a consistent synergistic pattern.

Table 4. Highest descriptive interaction means at 12 WAP

Trait	Combination with highest mean	Mean	Interaction inference
Plant height	M1N3 (40 g mycorrhiza + 10 g NPK)	18.64 cm	ns
Leaf number	M1N3 (40 g mycorrhiza + 10 g NPK)	10.36 leaves	ns
Leaf length	M2N3 (50 g mycorrhiza + 10 g NPK)	12.50 cm	ns
Stem diameter	M0N2 (0 g mycorrhiza + 6 g NPK)	6.95 mm	ns
Root volume	M1N1 (40 g mycorrhiza + 4 g NPK)	6.41 mL	ns

Note. These values are descriptive maxima only. Because the interaction was not significant (ns), they are not evidence of statistically superior treatment combinations.

One mechanistic explanation for a weak interaction is that readily available mineral phosphorus can reduce the host's relative dependence on mycorrhizal phosphorus acquisition. Phosphate transport in AM symbiosis is tightly regulated, and plants can shift between direct root uptake and the mycorrhizal uptake pathway according to nutrient conditions (Javot et al., 2007; Ferrol et al., 2019). Across field studies, phosphorus addition has frequently reduced mycorrhizal abundance (Treseder, 2004), while experimental work shows that AMF effects on productivity depend strongly on soil phosphorus availability (Yang et al., 2014). These mechanisms provide a plausible framework for the present non-significant interaction, but they remain an inference because soil available P and colonization were not measured in the experiment.

The non-significant interaction should also be considered against cocoa studies in which combinations with organic inputs produced synergy. Depari et al. (2018) evaluated cocoa-pod compost with NPK, Prasatyio et al. (2025) reported significant interactions between Asystasia compost and NPK for several cocoa seedling traits, and Hutauruk and Zahrah (2026) found interactive effects of empty-fruit-bunch bokashi and NPK Grower in a cocoa nursery. Those amendments modify substrate physical, chemical, and biological conditions in ways that a direct mycorrhiza-NPK pairing may not replicate. Consequently, the present finding is valuable precisely because it identifies a boundary condition: under the tested doses, medium, and 12-week observation period, adding mycorrhiza did not enhance the NPK response statistically.

Research contribution and implications

The principal contribution of this study is a stage-specific interpretation of an otherwise mostly non-significant factorial experiment. The data show that NPK effects appeared first and most consistently in leaf development: leaf number separated among fertilizer treatments from 4 WAP and remained separated through 12 WAP, while leaf length responded only from 4 to 8 WAP. Height, stem diameter, and root volume did not show parallel significant changes. This organ-specific sequence helps explain why final-only comparisons can understate early treatment effects and why nursery evaluation should combine repeated canopy measurements with structural and root indicators.

For practice, 10 g NPK/polybag generated the largest leaf number and the strongest early leaf-length response, but it should not be labeled a universal optimum because N2 and N3 were statistically similar for leaf number after 4 WAP and because structural traits did not improve significantly. For research, future experiments should quantify AMF colonization, soil available P, tissue N-P-K concentration, dry biomass, and seedling quality indices over a longer nursery period. Testing mycorrhiza under reduced NPK or P-limited conditions would also directly assess whether biological inoculation can maintain seedling quality while lowering mineral fertilizer input. Such a design would move from asking whether two inputs simply increase growth to asking whether AMF can improve nutrient-use efficiency under defined fertility constraints.

CONCLUSIONS

Cocoa seedlings displayed a clear stage- and organ-specific response to nursery nutrition. Mycorrhiza at 40-50 g/polybag did not significantly affect plant height, leaf number, leaf length, stem diameter, or root volume during the first 12 WAP. NPK fertilizer significantly affected leaf number from 4 through 12 WAP and leaf length at 4, 6, and 8 WAP, but it did not significantly alter final plant height, stem diameter, or root volume. The 10 g/polybag NPK treatment produced the largest leaf number at 12 WAP (9.96 leaves) and the largest leaf length during the significant early window (6.93 cm at 8 WAP). No mycorrhiza x NPK interaction was significant. Therefore, under the tested topsoil nursery conditions, mineral NPK produced an earlier and more detectable response than mycorrhizal inoculation, and that response was concentrated in leaf development rather than whole-seedling structure. Future work should extend the observation period and measure AMF colonization, substrate phosphorus, plant nutrient status, and biomass to determine whether mycorrhiza becomes beneficial under lower-fertility or reduced-NPK conditions.

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