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Synergistic Effects of *Arbuscular mycorrhizal* Inoculation and Soil Moisture on Leaf Nutrients and Physiological Performance in Cashew (*Anacardium occidentale L*) Biotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

A controlled greenhouse study was conducted during the 2020/2021 dry season at the Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria, to evaluate the effects of arbuscular mycorrhizal fungi (AMF) inoculation and soil moisture on nutrient acquisition and physiological performance of three cashew (*Anacardium occidentale* L.) biotypes. The experiment was arranged as a 3 × 3 × 2 factorial in a completely randomized design, comprising three biotypes (Jumbo, Medium and Madras), three soil moisture levels (40%, 60% and 80% field capacity), and two AMF treatments (inoculated and non-inoculated), with three replications. AMF inoculation significantly improved foliar nutrient concentration and nutrient uptake of N, P, K, Ca and Mg, although the magnitude of response varied with biotype and moisture regime. The highest nutrient uptake and foliar nutrient concentrations generally occurred in AMF-inoculated Jumbo seedlings at 80% field capacity. AMF inoculation also improved chlorophyll content, relative water content and stomatal conductance, with the highest physiological values recorded under 80%

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field capacity. Jumbo generally showed the strongest response, whereas Madras recorded comparatively lower values. The findings demonstrate the potential of AMF inoculation to improve nutrient acquisition and physiological performance of cashew seedlings under varying soil moisture conditions, with the response depending on the cashew biotype and moisture regime.

Keywords: *Arbuscular mycorrhizal fungi; cashew biotypes; water regimes; nutrient uptake; chlorophyll content; relative water content; stomatal conductance.*

1. Introduction

Cashew (*Anacardium occidentale* L.) is an important tropical perennial crop valued primarily for its nuts and its contribution to rural livelihoods and agricultural development. In Nigeria, cashew has become an important commercial crop and agrifood export, providing income opportunities along its production and value chains (FAO, 2025). However, cashew production is increasingly challenged by abiotic stresses, particularly water deficit. Drought can disrupt plant water balance, reduce photosynthetic activity and chlorophyll content, restrict nutrient uptake, and ultimately impair growth and productivity (Coulibaly, 2023). These effects make improved management of soil moisture and plant nutrient acquisition important for sustaining cashew establishment and productivity under water-limited conditions.*Arbuscular mycorrhizal* fungi form symbiotic associations with plant roots, enhancing nutrient (especially phosphorus and micronutrients) uptake and improving plant water relations and drought tolerance. By producing an extensive hyphal network beyond the root zone, AMF optimize resource acquisition under suboptimal conditions. The application of AMF has shown promising results in improving plant resilience to abiotic stresses, including limited water availability, in various crops (Shang *et al*., 2026). Recent evidence further indicates that AMF can improve plant growth and nutrient acquisition under stressful conditions, while meta-analytic findings show that established AM symbioses can lessen drought-related reductions in plant growth by supporting nutrient and water uptake (Singh *et al.,* 2024; Tang et al., 2024).

In particular, soil water content influences both the rate of *mycorrhizal* colonisation and the effectiveness of nutrient transfer from the soil to the plant. Conversely, AMF can mitigate the adverse impacts of drought by enhancing plant water relations, promoting root development, and improving internal biochemical processes. For perennial crops like cashew, the combined effect of AMF inoculation and soil moisture management may offer an eco - efficient solution to nutrient deficiencies and water stress (Tang *et al.,* 2022). However, this interaction remains underexplored in woody species compared to annual crops. Recent experimental evidence also indicates that AMF - mediated drought responses can vary with the environmental origin of the fungal inoculum, reinforcing the context dependence of symbiotic performance under water limitation (Decurgez *et al.,* 2026).

The leaf chemical composition reflects the internal nutrient and metabolic status of a plant, which is a useful indicator of physiological health. AMF- inoculated plants typically exhibit improved foliar nutrient content and biochemical traits. Yet, there is limited documentation on how different cashew genotypes respond to *mycorrhizal* inoculation under varying moisture regimes, especially in terms of nutrient uptake and foliar chemistry. Understanding this relationship is essential for developing sustainable production strategies in drought-prone environments.

1.1 Justification of the Study

As climate change intensifies the occurrence and severity of droughts, there is a growing imperative to develop ecologically sound strategies that bolster crop resilience and nutrient acquisition under water-limited conditions. *Arbuscular mycorrhizal* fungi represent a promising biofertiliser option, capable of enhancing nutrient uptake efficiency, promoting water-use optimisation, and improving overall plant performance while contributing to long-term soil health. However, the success of AMF symbiosis is modulated by both host plant genotype and prevailing environmental conditions, particularly soil moisture availability. Despite the agronomic relevance of cashew in tropical and subtropical areas, limited empirical data exist on genotype-specific responses to AMF inoculation under variable moisture regimes. This study addresses the critical knowledge gap by evaluating the interactive effects of AMF and soil moisture on physiological and nutrient dynamics across cashew biotypes. Insights from this work will contribute to the development of targeted, moisture-sensitive biofertilisation strategies for improving cashew productivity in drought-prone agroecosystems. More broadly, recent literature on plant growth-promoting microorganisms supports microbial biofertilizers as a strategy for improving nutrient acquisition and abiotic-stress tolerance, which is consistent with the biological nutrient-management rationale evaluated in the present study (Kumar *et al.,* 2025).

The objectives of this study therefore include:

a) To assess the impact of AMF inoculation on the foliar chemical composition of cashew under varying soil moisture levels.

b) To evaluate the effect of AMF and water regimes on nutrient uptake in cashew seedlings.

c) To investigate the interaction between cashew biotype, AMF inoculation, and soil moisture in influencing plant physiological performance.

d) To determine the most AMF- responsive cashew biotype under water-limited conditions for potential application in dryland cultivation.

2. Material and Methods

2.1 Study Location and Experimental Setup

The study was conducted during the 2020/2021 dry season in the greenhouse at the Headquarters of the Cocoa Research Institute of Nigeria (CRIN), Ibadan (7°10′N, 3°52′E), located within the tropical rainforest zone at an elevation of approximately 122 m above sea level.

The experiment was laid out as a 3 × 3 × 2 factorial experiment in a completely randomized design (CRD) with three replications, giving 18 treatment combinations and 54 experimental units. The three factors were cashew biotype (Jumbo, C_1; Medium, C_2; and Madras, C_3), soil moisture regime (40%, 60%, and 80% field capacity, designated F_1, F_2, and F_3, respectively), and AMF inoculation (M_1 = AMF- inoculated and M_0 = non-inoculated control).

Topsoil was collected from a cashew plantation, air-dried, and passed through a sieve to remove stones and other coarse materials. Ten kilograms of the prepared soil was weighed into each 10 - L plastic pot. Cashew seeds representing the three biotypes were sown in the respective pots. For the AMF treatments, 50 g of AMF inoculum was incorporated into the soil of the designated pots, while the corresponding non-inoculated pots received no AMF inoculum. The pots were maintained at the assigned soil moisture levels of 40%, 60%, and 80% field capacity throughout the experimental period. Seedlings were monitored for four months before harvest.

At harvest, the seedlings were carefully removed from the pots, washed to remove adhering soil, and separated into roots, stems, and leaves. The plant materials were weighed for fresh biomass determination and subsequently oven-dried to constant weight for dry biomass assessment.

2.2 Determination of Physiological Parameters

Chlorophyll content was measured on fully expanded leaves using a SPAD-502 chlorophyll meter following the procedure described by Markwell *et al.* (1995). Relative water content (RWC) was determined using fresh, turgid, and dry leaf weights according to the standard procedure:

$$RWC\ (\%) = TW - DWFW - DW \times 100$$

where :
FW is fresh weight, **TW** is turgid weight, and **DW** is dry weight.

Stomatal conductance was measured using a porometer and expressed as mmol $m^{-2}\ s^{-1}$.

2.3 Determination of Leaf Nutrient Concentration

For nutrient analysis, the oven-dried leaf samples were ground into a fine powder and subjected to laboratory analysis for N, P, K, Ca, and Mg using standard plant tissue analytical procedures based on IITA methods (Juo, 1978), as described in *Plant Analysis Handbook III* (Bryson *et al.*, 2014). Nutrient concentrations were expressed on a mg g^{-1} dry matter basis.

2.3.1 Determination of Nutrient Uptake

Nutrient uptake by the leaves was calculated from the nutrient concentration of the dried leaf tissue and the corresponding leaf dry biomass. For each nutrient, uptake was calculated as:

$$\text{Nutrient uptake (mg plant}^{-1}) = \text{Nutrient concentration (mg g}^{-1}) \times \text{Leaf dry matter (g plant)}$$

Thus, the nutrient concentration represented the amount of a nutrient present per unit dry weight of leaf tissue, whereas nutrient uptake represented the total amount of that nutrient accumulated in the leaves of each seedling.

2.4 Statistical Analysis

Data obtained from the experiment were subjected to analysis of variance (ANOVA) appropriate for a 3 × 3 × 2 factorial experiment, with cashew biotype, soil moisture regime and AMF inoculation as the main factors. The analysis was used to determine the significance of the main effects and their first- and second-order interactions among cashew biotype × soil moisture regime, cashew biotype × AMF inoculation, soil moisture regime × AMF inoculation, and cashew biotype × soil moisture regime × AMF inoculation. Where significant differences occurred, treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 5% probability level ($P \leq 0.05$). Standard error of the mean (SEM), standard error of difference (SEd), least significant difference (LSD) or critical difference (CD) at the 5% probability level, and coefficient of variation (CV) were calculated and presented where appropriate. The assumptions underlying ANOVA, including normality of residuals and homogeneity of variance, were assessed before interpretation of the results. Where necessary, appropriate data transformation was considered to satisfy the assumptions of ANOVA.

3. Results and Discussion

3.1 Effects of AMF Inoculation and Soil Moisture on Leaf Nutrient Concentration in Cashew

The concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in the leaves of the three cashew biotypes were significantly influenced by AMF inoculation, soil moisture regime, and their interactions (Table 1).

Table 1. Effects of AMF inoculation and soil moisture regimes on foliar nutrient concentrations of three cashew biotypes

Cashew biotype	AMF	Water regime (% FC)	N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (m/g)
Jumbo (C_1)	M_0	40	0.030^{h}	0.090^{m}	0.050^{h}	0.990^{i}	0.280^{k}
Jumbo (C_1)	M_0	60	0.060^{g}	1.233^{j}	0.090^{efg}	1.800^{g}	0.490^{hi}
Jumbo (C_1)	M_0	80	0.060^{g}	1.260^{j}	0.060^{gh}	1.500^{h}	0.420^{ij}
Jumbo (C_1)	M_1	40	0.153^{a}	2.950^{b}	0.210^{a}	4.380^{a}	1.240^{a}
Jumbo (C_1)	M_1	60	0.090^{de}	2.330^{de}	0.120^{cde}	2.550^{e}	0.720^{f}
Jumbo (C_1)	M_1	80	0.170^{a}	3.050^{a}	0.207^{a}	4.467^{a}	1.280^{a}
Medium (C_2)	M_0	40	0.060^{g}	1.233^{j}	0.090^{defg}	1.800^{g}	0.500^{h}
Medium (C_2)	M_0	60	0.070^{fg}	1.513^{i}	0.090^{defg}	2.340^{f}	0.610^{g}
Medium (C_2)	M_0	80	0.060^{g}	1.740^{h}	0.100^{def}	2.220^{f}	0.580^{g}
Medium (C_2)	M_1	40	0.120^{b}	2.380^{d}	0.160^{b}	3.240^{c}	0.943^{cd}
Medium (C_2)	M_1	60	0.100^{cd}	2.260^{ef}	0.160^{b}	3.090^{d}	0.883^{de}
Medium (C_2)	M_1	80	0.120^{b}	2.690^{c}	0.200^{a}	4.013^{b}	1.120^{b}
Madras (C_3)	M_0	40	0.050^{g}	0.090^{m}	0.070^{fgh}	1.470^{h}	0.400^{j}
Madras (C_3)	M_0	60	0.060^{g}	0.640^{l}	0.050^{h}	1.800^{g}	0.477^{hi}
Madras (C_3)	M_0	80	0.070^{fg}	0.980^{k}	0.080^{fgh}	2.220^{f}	0.590^{g}
Madras (C_3)	M_1	40	0.090^{de}	2.670^{c}	0.140^{bc}	3.880^{b}	1.060^{b}
Madras (C_3)	M_1	60	0.080^{ef}	2.240^{f}	0.120^{cd}	3.000^{d}	0.850^{e}
Madras (C_3)	M_1	80	0.110^{bc}	2.000^{g}	0.140^{bc}	3.300^{c}	0.970^{c}
SEM (±)			0.0061	0.0250	0.0097	0.0514	0.0239
SEd (±)			**0.0087**	**0.0353**	**0.0138**	**0.0728**	**0.0337**
CD/LSD ($P \leq 0.05$)			**0.0176**	**0.0718**	**0.0280**	**0.1478**	**0.0686**
CV (%)			**12.28**	**2.49**	**14.22**	**3.34**	

Note: C1 = Jumbo; C2 = Medium; C3 = Madras; M0 = non-inoculated control; M1 = AMF- inoculated treatment; F1 = 40% field capacity; F2 = 60% field capacity; F3 = 80% field capacity. Means followed by the same superscript letter within a column are not significantly different at P ≤ 0.05 according to Duncan's Multiple Range Test (DMRT). SEM = standard error of mean; SEd = standard error of difference; LSD/CD = least significant difference/critical difference at 5% probability; CV = coefficient of variation.

Across the biotypes and moisture regimes, AMF - inoculated seedlings generally recorded higher leaf nutrient concentrations than their corresponding non-inoculated controls. The highest concentrations of N, P, K, Ca, and Mg were recorded in the Jumbo biotype inoculated with AMF and maintained at 80% field capacity ($C_1F_3M_1$), with values of 0.17, 3.05, 0.21, 4.47, and 1.28 mg/g, respectively. These values were substantially higher than those recorded in the corresponding non-inoculated Jumbo seedlings at the same moisture level, indicating a strong response to AMF inoculation under adequate soil moisture. The lowest N, K, Ca, and Mg concentrations were recorded under the non-inoculated Jumbo treatment at 40% field capacity ($C_1F_1M_0$), with values of 0.03, 0.05, 0.99, and 0.28 mg/g, respectively. For P, the lowest concentration (0.09 mg/g) occurred under both $C_1F_1M_0$ and $C_3F_1M_0$. The response of leaf nutrient concentration to soil moisture was not uniformly linear across the treatments. Rather, nutrient concentrations varied according to the interaction between moisture regime, AMF inoculation, and cashew biotype. In several instances, AMF - inoculated seedlings at 80% field capacity recorded higher nutrient concentrations than those maintained at 40% and 60% field capacity, whereas some nutrients showed a temporary decline at 60% field capacity. This pattern indicates that the effect of soil moisture on nutrient concentration was dependent on both AMF status and cashew biotype.

The three biotypes also differed in their response to AMF inoculation and soil moisture. Jumbo generally exhibited the strongest nutrient response, particularly under AMF inoculation at 80% field capacity, while Medium and Madras showed comparatively lower concentrations for several nutrients. The significant interaction among biotype, soil moisture, and AMF inoculation indicates that nutrient acquisition was not determined by any single factor but by their combined influence. Overall, the results demonstrate that AMF inoculation enhanced leaf nutrient concentration in cashew seedlings, although the magnitude of the response depended on the biotype and prevailing soil moisture conditions.

The higher leaf concentrations of N, P, K, Ca and Mg recorded in AMF - inoculated cashew seedlings indicate that the fungal association improved nutrient acquisition under the conditions of the study. However, the magnitude of the response varied with cashew biotype and soil moisture regime. The strongest response was observed in the Jumbo biotype, particularly at 80% field capacity, where AMF - inoculated seedlings recorded 0.17 mg/g N, 3.05 mg/g P, 0.21 mg/g K, 4.47 mg/g Ca and 1.28 mg/g Mg. The corresponding values in the non-inoculated Jumbo seedlings at the same moisture level were considerably lower. The response across these nutrients suggests that the benefit of AMF was not restricted to a single nutrient**.** This observation is consistent with earlier work on cashew seedlings by Akanbi *et al.* (2013), which demonstrated the importance of improved nutrient supply for cashew seedling growth and development. The broader improvement in nutrient acquisition observed in the present study is also consistent with the role of the extraradical hyphal network in extending the volume of soil explored by roots and improving access to relatively immobile nutrients, particularly phosphorus (Chandrasekaran, 2020, 2022).

The response of phosphorus was particularly pronounced. Phosphorus concentration in AMF - inoculated seedlings was substantially higher than in the non-inoculated treatments under most of the moisture regimes. This agrees with the established contribution of AMF to P acquisition, as fungal hyphae can explore soil microsites beyond the immediate rhizosphere and facilitate the movement of P towards the root system. Improved P nutrition is important for energy transfer and root development and can consequently support the acquisition and utilisation of other nutrients by the developing plant (Chandrasekaran, 2022). The higher N and K concentrations recorded in several of the inoculated treatments further suggest that the effect of AMF extended beyond P nutrition. Thus, the association appears to have contributed to a broader improvement in the nutritional status of the seedlings.

The increases observed in Ca and Mg are also relevant to the physiological condition of the plants. Calcium contributes to cell wall stability, membrane integrity and cellular signalling, while Mg forms part of the chlorophyll molecule and is involved in photosynthesis and several enzyme-mediated processes. The relatively high concentrations of these nutrients in AMF - inoculated seedlings, particularly in Jumbo, suggest that the mycorrhizal association may have helped maintain a more favourable mineral balance during seedling development. Similar improvements in nutrient acquisition following AMF inoculation have been reported in plants exposed to drought or water-limited conditions (Chandrasekaran, 2022).

The response to soil moisture was not uniformly linear across the treatments. Although the highest nutrient concentrations in Jumbo occurred under AMF inoculation at 80% field capacity, some nutrients recorded lower values at 60% field capacity than at either 40% or 80% field capacity. For example, N concentration in AMF - inoculated Jumbo seedlings declined from 0.153 mg/g at 40% field capacity to 0.090 mg/g at 60%, before increasing to 0.170 mg/g at 80%. Similar fluctuations occurred for P, K, Ca and Mg. The results therefore point to a moisture-dependent response rather than a simple progressive increase in nutrient concentration with increasing soil moisture. Adequate soil water is important for nutrient dissolution, diffusion and movement towards roots, whereas water deficit can restrict root activity and nutrient transport. AMF may partly reduce these limitations by extending the volume of soil explored through fungal hyphae and improving access to both water and nutrients (Chandrasekaran, 2020; 2022).

The fact that AMF - inoculated seedlings maintained relatively higher nutrient concentrations even at 40% field capacity is important for cashew establishment under moisture-limited conditions. It suggests that the mycorrhizal association remained functional under reduced soil water availability rather than being restricted to well-watered conditions. AMF - mediated improvements in plant response to drought have been associated with enhanced water acquisition, improved root functioning and greater nutrient acquisition under water stress (Chandrasekaran, 2020; 2022). The present findings provide similar evidence in cashew seedlings, although the magnitude of the response differed among biotypes and nutrients.

The differences among the cashew biotypes were also evident. Jumbo generally recorded higher concentrations of the nutrients measured, with its most pronounced response occurring under AMF inoculation at 80% field capacity. Medium and Madras also responded to inoculation, but the magnitude of their responses varied with nutrient and moisture regime. The stronger response of Jumbo may be related to differences among the biotypes in root development, carbon allocation to the fungal partner, or the efficiency with which nutrients acquired through the mycorrhizal pathway are transferred and utilised by the host. Such differences are consistent with the view that the outcome of AMF symbiosis depends not only on the fungal partner but also on characteristics of the host plant (Chandrasekaran, 2022; Charakas & Khokhani, 2024). The significant treatment interactions observed in the present study further highlight the importance of considering host genotype when assessing the effectiveness of AMF inoculation.

The comparatively lower nutrient concentrations observed in some of the Medium and Madras treatments should not be taken to mean that these biotypes failed to respond to AMF. Rather, they indicate differences in the extent to which the three biotypes benefited from the association. Variation in AMF responsiveness among plant genotypes may arise from differences in root characteristics, host-fungus compatibility and the balance between the carbon cost of the symbiosis and the nutritional benefits obtained by the plant (Wu *et al.,* 2024). In the present study, the stronger response of Jumbo suggests that this biotype may have established a more effective association with the introduced AMF under the conditions of the experiment.

The differences in nutrient status were also consistent with the physiological responses observed in the seedlings. Higher concentrations of N, P, K, Ca and Mg provide a plausible nutritional basis for the greater chlorophyll content, relative water content and stomatal conductance recorded in AMF - inoculated seedlings. Nitrogen and magnesium are closely associated with chlorophyll formation and photosynthetic activity, while potassium plays an important role in stomatal regulation and plant water relations. The improved nutritional status following AMF inoculation may therefore have contributed to the better physiological functioning of the inoculated seedlings under the contrasting moisture regimes. Similar relationships between AMF inoculation, nutrient acquisition and plant performance under drought have been reported by Zaman *et al.* (2024).

The overall results confirm that AMF inoculation improved the nutritional status of cashew seedlings, though the extent of the response was shaped by the interaction between biotype and soil moisture. Jumbo showed the strongest response, particularly when inoculated seedlings were grown at 80% field capacity, and this biotype also retained a clear nutritional edge even under lower moisture availability. This points to AMF inoculation as a potentially useful biological approach to nutrient management in cashew nurseries, especially in settings where poor soil fertility coincides with irregular moisture supply.

The variation among biotypes also carries a practical implication: the choice of planting material may matter as much as the inoculation itself when designing AMF - based management strategies, since not all three biotypes benefited from the symbiosis to the same degree. Confirming whether these greenhouse-based responses hold up under field conditions will require further evaluation across a broader range of cashew genotypes, different soil types, and extended periods of moisture stress.

3.2 Leaf Nutrient Uptake as Influenced by AMF *inoculation*

Arbuscular mycorrhizal fungi (AMF) inoculation significantly influenced the uptake of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in the leaves of the three cashew biotypes (Table 2). The significant three-way interaction among cashew biotype, soil moisture regime and AMF inoculation indicates that the response of nutrient uptake depended on the particular combination of these factors.

In general, AMF - inoculated seedlings recorded higher nutrient uptake than the corresponding non-inoculated seedlings, although the magnitude of the response varied with biotype and soil moisture level. The highest uptake of all the nutrients was recorded in the Jumbo biotype inoculated with AMF and maintained at 80% field capacity ($C_1F_3M_1$), with N, P, K, Ca and Mg uptake of 1.38, 25.77, 1.83, 37.37 and 10.67 mg/g, respectively. In contrast, the lowest values for N, K, Ca and Mg occurred in non-inoculated Jumbo seedlings at 40% field capacity ($C_1F_1M_0$), with values of 0.28, 0.38, 8.22 and 2.32 mg/g, respectively. The lowest P uptake (0.72 mg/g) was also recorded under $C_1F_1M_0$.

The response to soil moisture was not uniform across all treatments. Although the highest nutrient uptake generally occurred at 80% field capacity under AMF inoculation, some nutrients fluctuated between the 40%, 60% and 80% moisture regimes. For example, N uptake in AMF - inoculated Jumbo seedlings was 1.35 mg/g at 40%, decreased to 0.75 mg/g at 60%, and increased to 1.38 mg/g at 80% field capacity. Similar variations occurred for the other nutrients, showing that nutrient uptake did not increase progressively with increasing soil moisture.

Differences were also evident among the cashew biotypes. Jumbo generally recorded the highest nutrient uptake, particularly under AMF inoculation, while Medium and Madras showed lower responses. At 80% field capacity under AMF inoculation, Medium recorded 1.01, 22.42, 1.69, 33.50 and 9.29 mg/g of N, P, K, Ca and Mg, respectively, compared with and 80.87, 16.66, 1.18, 27.50.08 mg/g, respectively, in Madras. These results show that the three biotypes differed in their capacity to acquire nutrients under the combinations of AMF inoculation and soil moisture tested.

Table 2. Effects of AMF inoculation and soil moisture regimes on nutrient uptake of three cashew biotypes (4MAA)

Cashew biotype	AMF	Water regime (% FC)	N uptake (mg/g)	P uptake (mg/g)	K uptake (mg/g)	Ca uptake (mg/g)	Mg uptake (mg/g)
Jumbo (C1)	M_0	40	0.28^{i}	0.72^{k}	0.38^{fj}	8.22^{f}	2.32^{f}
Jumbo (C1)	M_0	60	0.52^{gjk}	10.36^{h}	0.77^{di}	15.00^{ehi}	4.08^{eh}
Jumbo (C1)	M_0	80	0.50^{gk}	10.50^{h}	0.53^{ej}	12.50^{e}	3.50^{e}
Jumbo (C1)	M_1	40	0.54^{fjk}	10.20^{h}	0.72^{di}	15.00^{ehi}	4.16^{eh}
Jumbo (C1)	M_1	60	0.58^{f}	12.58^{g}	0.76^{di}	19.50^{dh}	5.08^{dh}
Jumbo (C1)	M_1	80	0.46^{h}	14.50^{f}	0.86^{dh}	18.50^{dhi}	4.83^{dh}
Medium (C2)	M_0	40	0.44^{h}	8.33^{i}	0.59^{ei}	12.25^{e}	3.33^{e}
Medium (C2)	M_0	60	0.54^{fjk}	5.37^{j}	0.42^{fj}	15.00^{ei}	4.00^{eh}
Medium (C2)	M_0	80	0.57^{fj}	8.16^{i}	0.69^{di}	18.50^{dh}	4.92^{dh}
Medium (C2)	M_1	40	1.35^{a}	24.60^{b}	1.78^{a}	36.50^{a}	10.33^{a}
Medium (C2)	M_1	60	0.75^{d}	19.45^{d}	1.02^{cg}	21.25^{d}	5.67^{d}
Medium (C2)	M_1	80	1.38^{a}	25.77^{a}	1.83^{a}	37.37^{a}	10.67^{a}
Madras (C3)	M_0	40	0.99^{b}	19.49^{d}	1.30^{b}	27.00^{c}	7.92^{cg}
Madras (C3)	M_0	60	0.87^{c}	18.91^{d}	1.30^{b}	25.75^{c}	7.33^{c}
Madras (C3)	M_0	80	1.01^{b}	22.42^{c}	1.69^{a}	33.50^{ag}	9.29^{b}
Madras (C3)	M_1	40	0.74^{d}	22.22^{c}	1.15^{bg}	32.35^{bg}	8.83^{bg}
Madras (C3)	M_1	60	0.63^{e}	18.70^{d}	0.99^{ch}	25.00^{c}	7.08^{c}
Madras (C3)	M_1	80	0.87^{c}	16.66^{e}	1.18^{b}	27.50^{c}	8.08^{cg}
SEM (±)			**0.0138**	**0.2377**	**0.0459**	**1.1534**	**0.2990**
SEd (±)			**0.0195**	**0.3361**	**0.0649**	**1.6312**	**0.4229**
LSD/CD (P ≤ 0.05)			**0.0396**	**0.6817**	**0.1316**	**3.3081**	**0.8576**
CV (%)			**3.31**	**2.76**	**7.97**	**8.97**	**8.37**

Notes: M_0 = non-inoculated control; M_1 = AMF-inoculated; F_1 = 40% field capacity; F_2 = 60% field capacity; F_3 = 80% field capacity. SEM = standard error of mean; LSD/CD = least significant difference/critical difference at $P \leq 0.05$; CV = coefficient of variation.

The higher nutrient uptake recorded in AMF-inoculated seedlings indicates that the mycorrhizal association contributed to more effective nutrient acquisition. This is particularly relevant under conditions where nutrient movement towards the root surface may be restricted. AMF produces an extensive extraradical hyphal network that extends beyond the immediate root zone, allowing the plant to explore a greater volume of soil and improving access to relatively immobile nutrients, especially phosphorus (Chandrasekaran*,* 2022; Duc *et al.,* 2023). The response observed in the present study therefore suggests that the contribution of AMF extended beyond phosphorus acquisition to the broader nutritional status of the cashew seedlings.

The moisture response, however, shows that the benefit of AMF should not be interpreted simply as increasing with soil water availability. The fluctuations recorded among the moisture regimes suggest that nutrient uptake was influenced by the combined effects of water availability, root activity and mycorrhizal functioning. Adequate moisture generally favours nutrient dissolution and movement in the soil, whereas restricted moisture can limit nutrient transport and root activity. The relatively high uptake maintained by inoculated seedlings at 40% field capacity nevertheless suggests that AMF helped sustain nutrient acquisition when water was limited. Similar benefits of AMF under drought or restricted water availability have been reported by Chandrasekaran (2022) and Duc *et al*. (2023).

The stronger response of Jumbo compared with Medium and Madras points to differences in the way the biotypes benefited from the symbiosis. Such differences may arise from variation in root development, host–fungus compatibility, carbon allocation to the fungal partner, and the efficiency with which nutrients acquired through the mycorrhizal pathway are transferred to the host plant (Chandrasekaran, 2022; Charakas & Khokhani, 2024; Wu *et al.,* 2024). The significant three-way interaction observed in this study further supports the view that AMF effectiveness cannot be considered independently of the host genotype and soil moisture conditions.

The findings are also in agreement with Wang *et al.* (2024), who reported that AMF symbiosis influenced nitrogen uptake and assimilation in *Populus cathayana* under drought conditions and contributed to improved drought tolerance. Their findings support the present observation that AMF can help maintain nutrient acquisition when water availability is restricted. However, the present results also show that the benefit was not uniform across the cashew biotypes or moisture regimes. AMF therefore appears to buffer the effect of moisture limitation rather than completely remove the constraint imposed by water deficit.

The nutritional advantage associated with AMF inoculation is important for early cashew establishment because adequate acquisition of N, P, K, Ca and Mg supports active growth and physiological functioning. The stronger response observed in Jumbo suggests that this biotype may be particularly responsive to AMF under the conditions tested. From a nursery management perspective, the findings support the use of AMF as a biological option for improving nutrient acquisition in cashew, particularly where soil moisture is variable. They also suggest that selection of responsive cashew biotypes should be considered alongside the choice of effective AMF inoculants when developing mycorrhiza - based nursery management practices.

3.3 AMF Infection Rates in Cashew Biotypes

Table 3 shows that AMF inoculation (M_1) consistently increased root colonisation in the cashew biotypes compared with the non-inoculated control (M_0), resulting in mean colonisation rates of 59.6% and 47.0%, respectively. The higher colonisation observed in inoculated seedlings ($P < 0.05$) agrees with recent reports that AMF inoculation promotes successful root colonisation and improves plant growth, biomass accumulation, and nutrient acquisition across a wide range of crop species (Wu *et al.,* 2024; Zaman *et al.*, 2024).

Cashew biotypes responded differently, with Jumbo (C_1) showing the highest increase, peaking at 65% ($C_1F_1M_1$), while Madras (C_3) recorded more moderate gains (57–59% under M_1 vs. 39–56% under M_0). These genotype-specific responses agree with Balogoun *et al.* (2014), who observed variability among cashew cultivars in Benin. Soil moisture also influenced colonisation, consistent with Sun *et al.* (2022), who found that drought reduced AMF richness and colonization.

The enhanced colonisation under M_1 reflects the role of AMF in improving plant resilience, as Shang *et al*. (2026) also showed improved biomass and nutrient uptake under drought stress with AMF inoculation. However, working in a cold-temperate grassland, Fu *et al.* (2022) found that extreme drought altered *arbuscular mycorrhizal* fungal community composition, with colonization and richness shifting in response to soil moisture availability.

Table 3. Effects of AMF on the rate of root infection (%) of Cashew at 4WAA

Treatments	Infection Rate (%)
$C_1F_1M_0$	40
$C_1F_2M_0$	47
$C_1F_3M_0$	53
$C_2F_1M_0$	51
$C_2F_2M_0$	48
$C_2F_3M_0$	46
$C_3F_1M_0$	56
$C_3F_2M_0$	44
$C_3F_3M_0$	39
$C_1F_1M_1$	65
$C_1F_2M_1$	60
$C_1F_3M_1$	61
$C_2F_1M_1$	60
$C_2F_2M_1$	59
$C_2F_3M_1$	61
$C_3F_1M_1$	59
$C_3F_2M_1$	57
$C_3F_3M_1$	58

The infection rates under M_0 showed variable trends with field capacity, increasing in C_1 ($F_1 < F_2 < F_3$) but declining in C_3 ($F_1 > F_2 > F_3$). Under M_1, colonisation became more stable across moisture regimes, suggesting that mycorrhizal inoculation reduced moisture sensitivity. This agrees with Assih *et al.* (2022), who reported that AMF mitigates water deficit effects in cashew seedlings, and with Shang *et al*. (2026) who found that AMF enhanced biomass and nutrient uptake under drought stress. The results indicate that AMF inoculation consistently improves root colonisation across biotypes and moisture levels, buffering against variability in soil water. Similar effects were observed by Jadrane *et al.* (2021) in *Ceratonia siliqua* under drought conditions. Notably, Jumbo cashew (C_1) was the most responsive to AMF, particularly at 40% field capacity, highlighting its suitability for AMF - assisted growth under limited water. This supports Li *et al.* (2023), who showed that *AMF* enhanced microbial activity and drought tolerance in oat, with genotypic differences influencing responsiveness under water stress.

3.4 Physiological Responses of Cashew Biotypes to AMF Inoculation and Soil Moisture Levels

The physiological responses of the three cashew biotypes were significantly affected by AMF inoculation, soil moisture regime, and biotype (Table 4). Chlorophyll content, relative water content (RWC), and stomatal conductance all showed significant differences among the AMF treatments, moisture regimes, and cashew biotypes ($P < 0.001$). However, the interactions among biotype, soil moisture regime, and AMF inoculation were not significant for any of the physiological traits measured. This suggests that the observed changes were mainly due to the separate effects of AMF inoculation, soil moisture, and biotype.

In general, AMF-inoculated seedlings recorded higher chlorophyll content, RWC, and stomatal conductance than the non-inoculated seedlings. The highest values were observed in the Jumbo biotype (C_1) at 80% field capacity with AMF inoculation ($C_1F_3M_1$), recording 49 SPAD for chlorophyll content, 81% RWC, and 212 mmol m^{-2} s^{-1} for stomatal conductance. In contrast, the lowest values were recorded in the Madras biotype (C_3) at 40% field capacity without AMF inoculation ($C_3F_1M_0$), with 37 SPAD, 68% RWC, and 160 mmol m^{-2} s^{-1}, respectively. Across the different moisture regimes, seedlings receiving AMF inoculation generally maintained higher values for the three physiological traits than the non-inoculated seedlings.

Table 4. Physiological responses of three cashew biotypes to AMF inoculation and soil moisture regimes

Cashew biotype	AMF	Soil moisture (% FC)	Treatment	Chlorophyll content (SPAD)	Relative water content (%)	Stomatal conductance (mmol m^{-2} s^{-1})
Jumbo (C_1)	M_0	40	$C_1F_1M_0$	41^{bfg}	73^{cghi}	180^{d}
Jumbo (C_1)	M_0	60	$C_1F_2M_0$	42^{bef}	75^{bfgh}	190^{c}
Jumbo (C_1)	M_0	80	$C_1F_3M_0$	45^{adef}	77^{bef}	200^{b}
Jumbo (C_1)	M_1	40	$C_1F_1M_1$	45^{adef}	77^{bef}	192^{c}
Jumbo (C_1)	M_1	60	$C_1F_2M_1$	47^{ade}	79^{ae}	202^{b}
Jumbo (C_1)	M_1	80	$C_1F_3M_1$	**49^{a}**	**81^{a}**	**212^{a}**
Medium (C_2)	M_0	40	$C_2F_1M_0$	39^{bg}	70^{di}	170^{e}
Medium (C_2)	M_0	60	$C_2F_2M_0$	41^{bfg}	72^{chi}	180^{d}
Medium (C_2)	M_0	80	$C_2F_3M_0$	43^{bdf}	74^{cfgh}	190^{c}
Medium (C_2)	M_1	40	$C_2F_1M_1$	43^{bdf}	74^{cfgh}	182^{d}
Medium (C_2)	M_1	60	$C_2F_2M_1$	44.67^{adef}	76^{befg}	192^{c}
Medium (C_2)	M_1	80	$C_2F_3M_1$	47^{ad}	78^{be}	202^{b}
Madras (C_3)	M_0	40	$C_3F_1M_0$	37^{cg}	68^{d}	160^{f}
Madras (C_3)	M_0	60	$C_3F_2M_0$	39^{bg}	70^{di}	170^{e}
Madras (C_3)	M_0	80	$C_3F_3M_0$	41^{bfg}	72^{chi}	180^{d}
Madras (C_3)	M_1	40	$C_3F_1M_1$	41^{bfg}	72^{chi}	172^{e}
Madras (C_3)	M_1	60	$C_3F_2M_1$	43^{bdf}	74^{cfgh}	182^{d}
Madras (C_3)	M_1	80	$C_3F_3M_1$	45^{adef}	76^{befg}	192^{c}
SEM ±				**1.059**	**0.816**	**1.130**
SEd ±				**1.498**	**1.155**	**1.599**
LSD/CD (5%)				**3.038**	**2.342**	**3.242**
CV (%)				**4.27**	**1.90**	**1.05**

Notes: M_0 = non-inoculated control; M_1 = AMF-inoculated; F_1 = 40% field capacity; F_2 = 60% field capacity; F_3 = 80% field capacity. SEM = standard error of mean; LSD/CD = least significant difference/critical difference at $P \leq 0.05$; CV = coefficient of variation.

The higher chlorophyll content, relative water content (RWC), and stomatal conductance recorded in the AMF-inoculated seedlings suggest that the mycorrhizal association contributed to better physiological performance in the cashew seedlings. The response was observed across the soil moisture levels used in the study, showing that the presence of AMF was generally beneficial to the plants. This agrees with earlier reports that AMF can improve plant water relations, photosynthetic activity, and stomatal regulation, particularly when plants are exposed to drought or limited water supply (Chandrasekaran, 2022; Duc *et al.*, 2023; Chandrasekaran, 2024; Guo *et al.,* 2024).

Soil moisture also played an important role in the physiological performance of the seedlings. The generally higher chlorophyll content, RWC, and stomatal conductance recorded at 80% field capacity indicate that adequate water availability favoured better leaf hydration and physiological activity. At 40% field capacity, the physiological values were generally lower, but AMF - inoculated seedlings still tended to record higher values than their non-inoculated counterparts. This suggests that AMF may have helped the seedlings maintain some level of physiological activity when soil water was limited. However, the biotype × moisture, biotype × AMF, moisture × AMF, and three-way interactions were not significant. The responses should therefore be understood mainly in terms of the individual effects of AMF inoculation, soil moisture, and biotype rather than as evidence of a significant interaction among these factors.

The three cashew biotypes also showed differences in their physiological performance. Jumbo (C_1) generally recorded higher chlorophyll content, RWC, and stomatal conductance than Medium (C_2) and Madras (C_3), while Madras generally had the lowest values. The highest numerical values occurred in Jumbo under AMF inoculation at 80% field capacity, where chlorophyll content, RWC, and stomatal conductance reached 49 SPAD, 81%, and 212 mmol m^{-2} s^{-1}, respectively. On the other hand, Madras recorded the lowest values under non-inoculated conditions at 40% field capacity. The differences among the biotypes may be related to their ability to maintain leaf water status and physiological activity under different moisture conditions and following AMF inoculation.

The changes observed in the three physiological traits are also important for understanding how the seedlings responded to the treatments. Chlorophyll content provides an indication of the condition of the photosynthetic apparatus, while RWC reflects the water status of the leaves. Stomatal conductance, on the other hand, provides an indication of the ability of the plants to regulate gas exchange. The higher values recorded in AMF - inoculated seedlings therefore suggest that these plants were better able to maintain their physiological functions under the conditions of the experiment. This observation is supported by Abdelaal *et al.* (2024), who reported that AMF inoculation reduced drought-related reductions in chlorophyll a and b, PS II efficiency, and RWC compared with non-inoculated plants exposed to drought. Although the crop species and experimental conditions differed, their findings provide useful support for the physiological response observed in the present study.

The better physiological performance of Jumbo, particularly under AMF inoculation and adequate soil moisture, suggests that this biotype was better able to maintain physiological activity under the conditions tested. This finding is useful for understanding the early response of the three cashew biotypes, but it should not be taken as conclusive evidence that Jumbo is more drought-tolerant under field conditions. The present experiment was conducted under controlled conditions and over a relatively short period. Further studies under field conditions, particularly with longer periods of water stress, would help to determine whether the advantage observed in Jumbo is maintained during cashew establishment and subsequent growth.

4. Summary and Conclusion

This study assessed the effects of *Arbuscular mycorrhizal* fungi (AMF) inoculation and soil moisture on root colonization, foliar nutrient concentration, nutrient uptake, and selected physiological traits of three cashew biotypes (Jumbo, Medium, and Madras) grown at 40%, 60%, and 80% field capacity. The experiment was conducted using a 3 × 3 × 2 factorial arrangement involving three cashew biotypes, three soil moisture regimes, and two AMF treatments. AMF inoculation increased root colonization and generally improved the concentration and uptake of N, P, K, Ca, and Mg in the seedlings. It also improved chlorophyll content, relative water content, and stomatal conductance. The physiological traits were significantly affected by biotype, soil moisture, and AMF inoculation, whereas their interactions were not significant. Jumbo generally recorded the highest nutrient and physiological values, while Madras tended to record the lowest. The highest physiological values were obtained in AMF - inoculated Jumbo seedlings at 80% field capacity, with 49 SPAD, 81% relative water content, and 212 mmol m^{-2} s^{-1} stomatal conductance. These findings indicate that AMF inoculation improved nutrient acquisition and physiological performance of young cashew seedlings under the moisture conditions studied. The stronger performance of Jumbo also suggests that it may be more responsive to AMF inoculation than the other biotypes evaluated. However, because the study was conducted under greenhouse conditions for four months, the findings mainly reflect early seedling performance and may not fully represent field conditions. Further field studies involving more cashew genotypes, locations, and longer periods are therefore needed to establish whether the observed benefits of AMF persist during tree establishment and contribute to growth, nutrient-use efficiency, moisture-stress tolerance, and ultimately nut yield.

5. Limitations

The study was conducted under greenhouse conditions for four months and included three cashew biotypes, three soil-moisture levels, and an inoculated versus non-inoculated AMF treatment. Consequently, the findings represent early seedling responses under controlled conditions and cannot be directly extrapolated to long-term field establishment, drought tolerance, or nut yield. Responses may also vary with soil type, AMF inoculum composition, genotype, and the duration or severity of moisture stress. Multi-location field studies with more cashew genotypes and extended evaluation periods are therefore needed.

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Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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