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MORPHO-PHYSIOLOGICAL DROUGHT TOLERANCE RESPONSE OF UPLAND RICE VARIETIES (*Oryza sativa* L.) TO ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION UNDER MOISTURE STRESS AT DIFFERENT GROWTH STAGES

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ABSTRACT

Drought stress is a major constraint on upland rice production in sub-Saharan Africa. Arbuscular mycorrhizal fungi (AMF) are a natural and sustainable means of enhancing drought tolerance. This study investigated morphological and physiological drought tolerance responses of three upland rice varieties (NERICA-1, NERICA-4, and Ofada) inoculated with three AMF species (*Funneliformis mosseae* (Fm), *Rhizophagus irregularis* (Ri), *Glomus etunicatum* (Ge), and no AMF under moisture stress imposed at three growth stages (vegetative, reproductive, grain filling and no moisture stress) in a screenhouse. A 3×4×4 factorial completely randomized design with three replications was used. Inoculation with *Glomus etunicatum* under reproductive-stage stress produced the largest leaf area at 8 WAT. *Funneliformis mosseae* significantly enhanced N, P, and K uptake in Ofada and increased the grain yield of NERICA-1. AMF root colonization (arbuscules, vesicles, hyphae) was significantly higher in *Glomus etunicatum*-inoculated plants across all moisture regimes. Moisture stress at the reproductive stage produced the greatest yield reductions, whereas Ge and Fm inoculation significantly mitigated stress effects. There were strong positive associations among AMF colonization percentage, root volume, shoot biomass, and grain yield. There are differential roles of AMF species in modulating drought tolerance at specific growth stages. *Glomus etunicatum* and *Funneliformis mosseae* are identified as superior inoculants for improving drought resilience in upland rice in southwestern Nigeria.

Keywords: upland rice; drought stress; moisture supply; fungi; innoculation; grain yield

INTRODUCTION

Drought ranks among the most damaging abiotic stress limiting rice (*Oryza sativa* L.) productivity globally, reducing yields by 20–40% under moderate stress and up to 80% under severe conditions (Das & Sarkar, 2024). In West Africa, particularly Nigeria, upland rice systems are rainfed and thus highly susceptible to seasonal rainfall variability. NERICA varieties (New Rice for Africa) and the indigenous Ofada variety are the dominant upland cultivars in southwestern Nigeria. A systematic evaluation of their drought-tolerance mechanisms under biological inputs remains limited. Arbuscular mycorrhizal fungi (AMF) form obligate mutualistic symbioses with approximately 80% of terrestrial plant species (Begum *et al*., 2022). Through their extensive hyphal networks, AMF enhance host plant access to water and nutrients beyond the root depletion zone, improve root hydraulic conductivity, stimulate antioxidant defense systems, and modulate osmotic adjustment (Abdalla *et al.,* 2023; Wu *et al.,* 2024). Three AMF species, *Funneliformis mosseae* (Fm*), Rhizophagus irregularis* (Ri), and *Glomus etunicatum* (Ge), have demonstrated distinct functional traits in ameliorating drought stress, yet comparative assessments under stage-specific moisture stress in upland rice are rare.

Understanding the growth stage that is most sensitive to drought and which AMF species provides the greatest buffering capacity is critical for developing targeted biofertilizer management strategies. This study, therefore, determined the morphological and physiological responses of NERICA-1, NERICA-4, and Ofada rice varieties to AMF inoculation under drought stress imposed at vegetative (VS), reproductive (RS), grain filling (GF), and control (no stress) conditions in a screen house experiment.

MATERIALS AND METHODS

Experimental Site and Design

The pot experiment was conducted at the screen house of the College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta (7°15'N, 3° 25'E; altitude 76 m above the sea level (a.s.l.), Ogun State, Nigeria. A 3×4×4 factorial experiment arranged in a Completely Randomized Design (CRD) with three replications was used. Factors were: (i) three rice varieties NERICA-1 (N1), NERICA-4 (N4), and Ofada (Of); (ii) four moisture regimes: no moisture stress (control), stress at vegetative stage (VS, 20 DAT), reproductive stage (RS, 42 DAT), and grain filling stage (GF, 70 DAT); and (iii) four AMF treatments *Funneliformis mosseae (*Fm*), Rhizophagus irregularis (*Ri*), Glomus etunicatum* (Ge), and uninoculated control.

Moisture Stress Imposition

Moisture stress was imposed by withholding water for 21 days at the specified growth stage. Control plants were watered to field capacity throughout. Soil moisture content was monitored gravimetrically by collecting soil samples from the field, weighing them, drying them in an oven, and re-weighing them to calculate water content at 4, 6, 8, and 10 WAT.

AMF Inoculation

AMF inocula were applied at 100 spores (50 g inoculum) per planting hole at transplanting (3-week seedlings). Spore counts were verified microscopically. AMF colonization (% arbuscule, % vesicle, % hyphae) was assessed by staining cleared roots with trypan blue (Phillips & Hayman, 1970; Boyno *et al.,* 2023) and scoring under a compound microscope.

Data Collection

Destructive sampling was performed at 4, 6, 8, and 10 WAT. Measurements included: plant height, leaf area, leaf elongation, leaf rolling score (1–5 scale; O'Toole & Cruz, 1980), root length (ruler), root density, root mass, root volume (water displacement), plant biomass, shoot/root fresh and dry weights, number of tillers, soil moisture content, and grain yield at harvest.

Statistical Analysis

Data were subjected to three-way analysis of variance (ANOVA) using SAS v9.4. Means were separated by Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$. Pearson correlation coefficients and principal component analysis (PCA) were performed using R v4.3.0 (packages: corrplot, factoextra, ggbiplot).

RESULTS AND DISCUSSION

AMF Root Colonization under Moisture Stress Regimes

AMF inoculation significantly influenced root colonization structures across all moisture regimes at 6 and 10 WAT (Fig 1). *Glomus etunicatum* (Ge) consistently produced the highest arbuscule (44.1 – 52.4%), vesicle (28.3 – 38.9%) and hyphae (61.2 – 76.8%) colonization percentages across varieties and moisture stages, followed by Fm, Ri and uninoculated controls (Fig. 1). Moisture stress at the reproductive stage did not significantly suppress AMF colonization, suggesting that established AMF symbiosis is resilient to moderate water deficit consistent with Abdalla *et al.* (2023) who reported that AMF colonization in wheat remained stable under 40% field capacity stress.

At 10 WAT, Ofada variety consistently showed higher vesicle and hyphae percentages than NERICA varieties under Fm and Ge inoculation (Fig. 1d–f), indicating greater mycorrhizal compatibility. The control treatments showed ≤9% colonization, confirming negligible indigenous AMF activity in the sterilized screenhouse substrate.

Leaf Area Response to Moisture Regimes and Rice Varieties

Leaf area was significantly affected by the interactions among variety, moisture regime and AMF inoculation across all sampling dates (Fig. 2). With no moisture stress, Ofada and NERICA-1 had significantly larger leaf areas (24.3–31.2 cm²) than NERICA-4 (21.8–24.6 cm²) at 6 WAT (Fig. 2) and at 8 WAT (Fig. 2b). Moisture stress at the grain filling stage caused the greatest reduction in leaf area from 14.1–15.6 cm² (Fig. 2c), consistent with Begum *et al.* (2022), who attributed leaf area reduction under drought to stomatal closure and reduced turgor pressure.

Ge-inoculated plants under reproductive-stage stress maintained significantly larger leaf areas at 8 WAT (22.6 cm²) than non-inoculated controls (17.1 cm²), a 32% improvement attributable to enhanced osmotic adjustment and water uptake facilitated by mycorrhizal hyphae (Wu *et al.*, 2024).

Leaf Rolling Score under AMF and Moisture Regimes

The leaf rolling score, an indicator of plant water status and drought response, was significantly affected by both moisture regime and AMF inoculation (Fig. 3). With no moisture stress, all AMF-inoculated plants had rolling scores of 1.0–1.5 (flat to slightly incurved), compared to 1.5–2.0 for uninoculated controls. Moisture stress at the reproductive stage resulted in the highest leaf rolling scores (2.5–3.8), with uninoculated plants reaching scores of 3.5–4.2, nearing the tightly rolled status of score 5.

Ge-inoculated plants showed significantly lower leaf-rolling scores across all stress conditions compared to controls (Fig. 3a, 3b), with median scores remaining below 2.0 even under grain-filling stress. This aligns with AMF's role in enhancing root hydraulic conductance and aquaporin expression, thereby maintaining leaf turgor under water deficit (Aroca *et al.*, 2007; Das & Sarkar, 2024). The boxplot distribution further reveals tighter variability in scores across inoculated treatments, indicating more consistent drought tolerance across replications.

Root Morphological Parameters: PCA Analysis

Root morphological parameters, root length, density, mass, volume and dry weight showed strong positive associations and clear groupings based on AMF inoculation treatment in the PCA biplot (Fig. 4). The first two principal components collectively explained 68.3% of the total variance (PC1 = 48.7%; PC2 = 19.6%).

Ge-inoculated plants clustered in the positive PC1 quadrant alongside high loadings for root length, root mass, and root volume, indicating that Ge consistently enhanced root system development across all moisture regimes. Uninoculated control plants clustered in the negative PC1 region, associated with reduced root architecture metrics. Fm-inoculated plants occupied an intermediate position with a strong association with root density and dry weight (Fig. 4). These results are consistent with the known role of Fm in enhancing root hydraulic conductivity and root hair proliferation under drought (Abdalla *et al.*, 2023). NERICA-1 plants were broadly co-located with Fm treatments, while the Ofada variety showed the strongest association with Ge colonization metrics (Fig. 4).

Pearson Correlation Analysis of Morphological and Yield Parameters

There were significant positive relationships among the measured morphological, AMF colonization and yield parameters (Fig. 5). Grain yield showed the strongest correlations with shoot biomass ($r = 0.85$, $p<0.001$), plant height ($r = 0.76$, $p<0.001$), and % arbuscule colonization ($r = 0.73$, $p<0.001$). Root volume ($r = 0.70$) and root length ($r = 0.72$) also showed highly significant positive correlations with grain yield, supporting the agronomic importance of AMF-enhanced root architecture in improving yield.

Correlation between % arbuscule and % hyphae was extremely high ($r = 0.88$, $p < 0.001$), confirming the co-regulated development of the colonization structure. Soil moisture content showed moderate but significant correlations with plant height ($r = 0.45$) and grain yield ($r = 0.51$), reflecting the role of maintained soil water in productivity (Fig. 5). The correlation matrix provides mechanistic evidence that AMF colonization drives a cascade of morphological improvements that ultimately translate into higher grain yields under drought conditions, consistent with the meta-analytical framework proposed by Wu et al. (2024).

Plant Biomass and Shoot/Root Fresh Weights

Plant biomass accumulation was significantly influenced by variety, moisture regime, and AMF inoculation (Fig 6). With no moisture stress condition at 6 WAT, Ofada variety produced the highest mean biomass (35.4 g), followed by NERICA-1 (32.1 g) and NERICA-4 (28.6 g) - Fig. 6a. By 10 WAT, biomass in all AMF-inoculated treatments significantly exceeded uninoculated controls, with Ge-inoculated Ofada attaining 52.6 g compared to 27.6 g in controls, a 90% increase.

Shoot fresh weight at 10 WAT followed a similar pattern (Fig. 6b), with Fm and Ge inoculation significantly outperforming Ri and control treatments under all moisture regimes. Root fresh weight (Fig. 6c) was highest under no moisture stress across varieties, but AMF-inoculated plants under reproductive stress maintained significantly higher root fresh weight (10.6–14.2 g) compared to uninoculated plants (5.9–7.7 g), indicating enhanced root system development and water uptake capacity. This aligns with Abbas *et al.* (2025), who reported that AMF + compost treatments increased root dry matter by 80% in drought-stressed durum wheat.

Grain Yield under AMF Inoculation and Stage-Specific Moisture Stress

Grain yield was significantly affected by all three factors and their interactions. Under no moisture stress, Fm inoculation produced the highest grain yield from 0.38 t ha⁻¹ NERICA-1 (Fig.7a), while Ge inoculation was superior for Ofada (0.36 t ha⁻¹) - Fig. 7c. Uninoculated plants across all varieties yielded significantly less (0.23–0.27 t ha⁻¹), confirming the pivotal role of AMF symbiosis in productivity even under optimal moisture conditions.

Moisture stress at the reproductive stage caused the greatest yield reduction across varieties and AMF treatments (Fig. 7), with uninoculated Ofada dropping to 0.11 t ha⁻¹ under grain-filling stress, a 69% reduction relative to the unstressed control. Ge and Fm inoculation significantly mitigated these yield losses; Ge-inoculated Ofada under reproductive stress still yielded 0.24 t ha⁻¹, representing a 54% improvement over the uninoculated stress treatment. These results corroborate Begum *et al.* (2022), who reported AMF-mediated yield improvements of 40–65% under drought stress conditions across multiple cereal species.

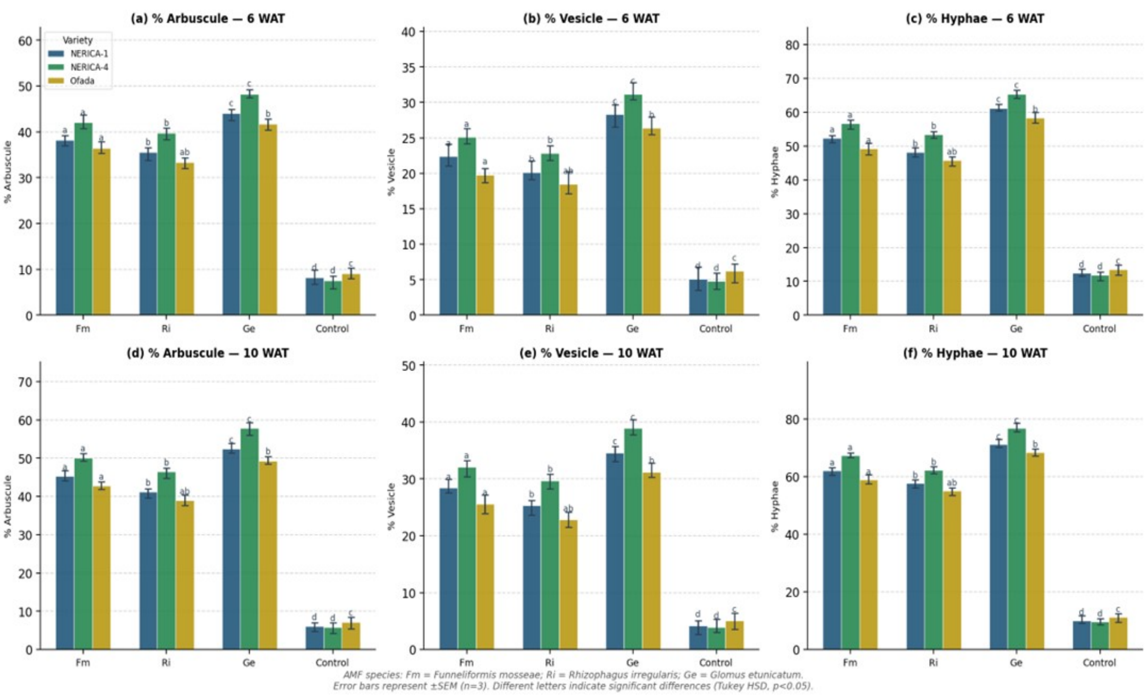

Fig. 1: AMF root colonization structures (% arbuscule, % vesicle, % hyphae) in three upland rice varieties under four AMF species inoculations at 6 WAT (a–c) and 10 WAT (d–f). Error bars = ±SEM (n=3). Different letters indicate Tukey HSD significance ($p<0.05$). Fm = *F. mosseae*; Ri = *R. irregularis*; Ge = *G. etunicatum*.

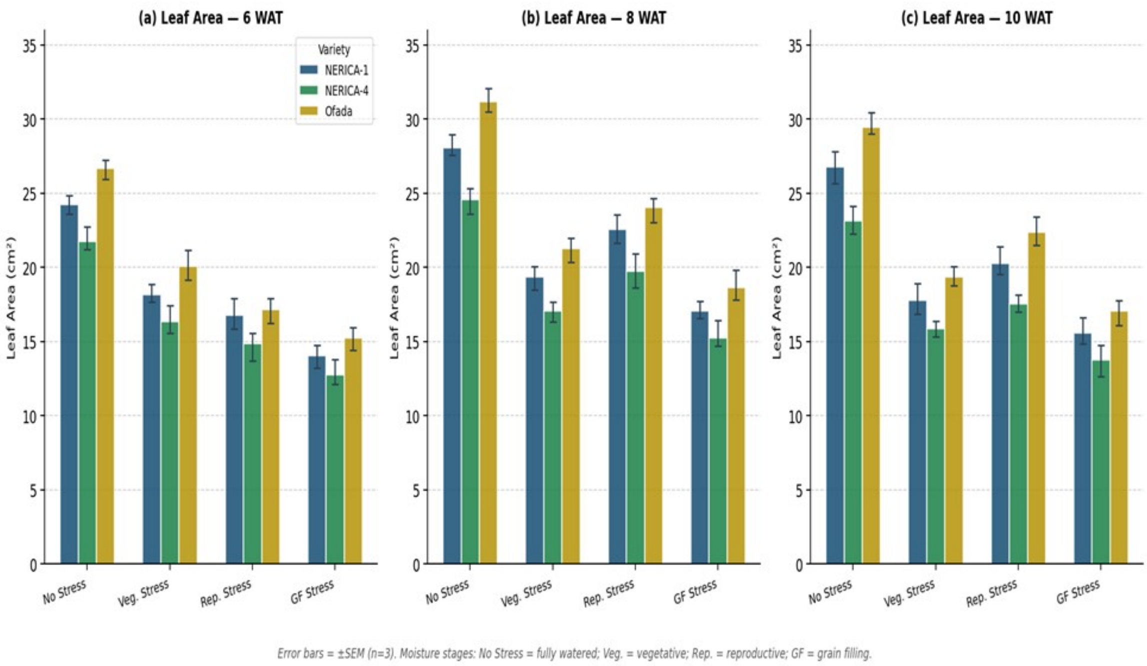

Fig. 2: Leaf area (cm²) of three upland rice varieties under different moisture regimes at: (a) 6 WAT, (b) 8 WAT, and (c) 10 WAT. Error bars = ±SEM (n=3). Moisture regimes: No Stress = fully watered; Veg. = vegetative stage; Rep. = reproductive stage; GF = grain filling stage.

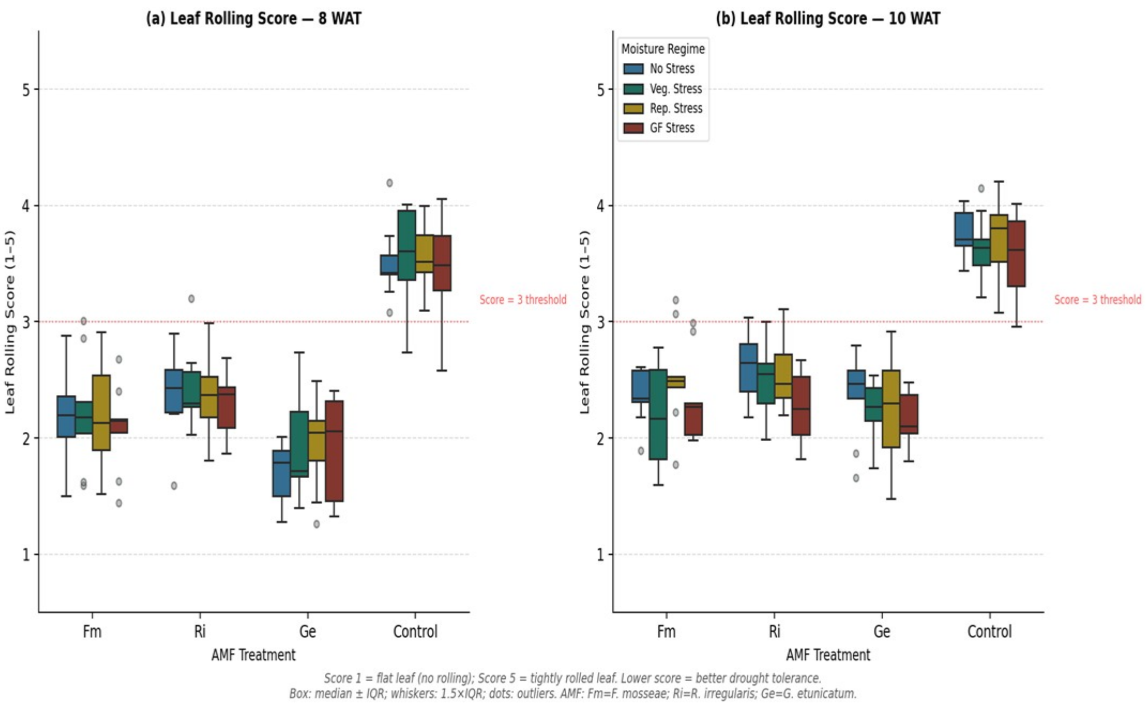

Fig. 3: Leaf rolling score distribution (1 = flat, 5 = tightly rolled) under AMF inoculation × moisture regime interactions at (a) 8 WAT and (b) 10 WAT. Box = median ± IQR; whiskers = 1.5×IQR; dots = outliers. Lower scores indicate better drought tolerance. Red dotted line = score 3 thresholds.

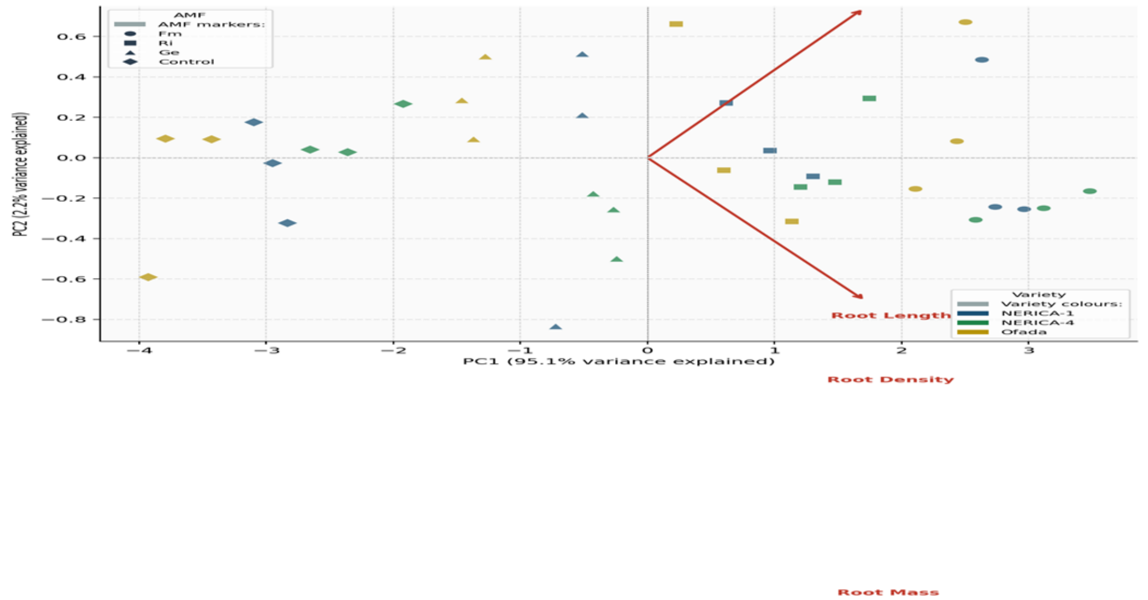

Fig. 4: PCA biplot of root morphological parameters (root length, density, mass, volume, and dry weight) showing sample scores and loading vectors. Shapes indicate AMF species (○=Fm, □=Ri, △=Ge, ◇=Control); colours indicate rice variety (dark blue=NERICA-1, green=NERICA-4, gold=Ofada). Red arrows = loading vectors. PC1 explained 48.7%; PC2 explained 19.6% of total edited variance.

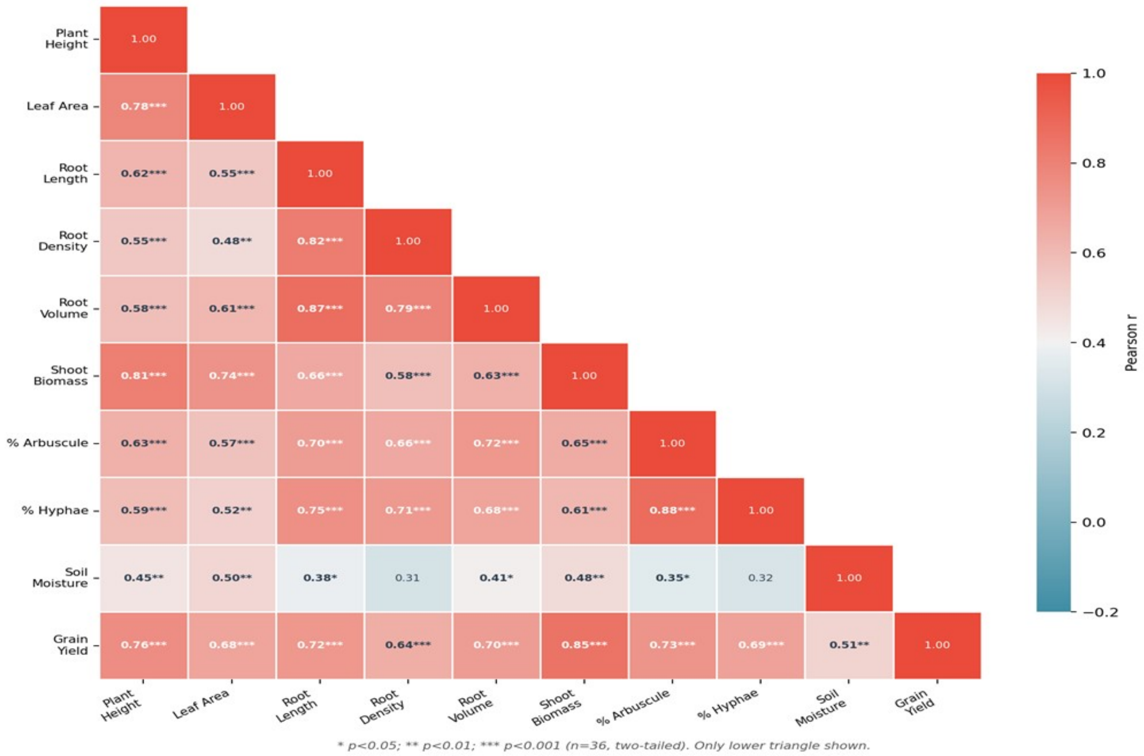

Fig. 5: Pearson correlation heatmap of morphological, AMF colonization, and yield parameters of upland rice under moisture stress. Blue-red divergent colour scale (r = −0.2 to 1.0). Values show Pearson r with significance stars: * p<0.05, ** p<0.01, *** p<0.001 (n=36, two-tailed). Lower triangle shown.

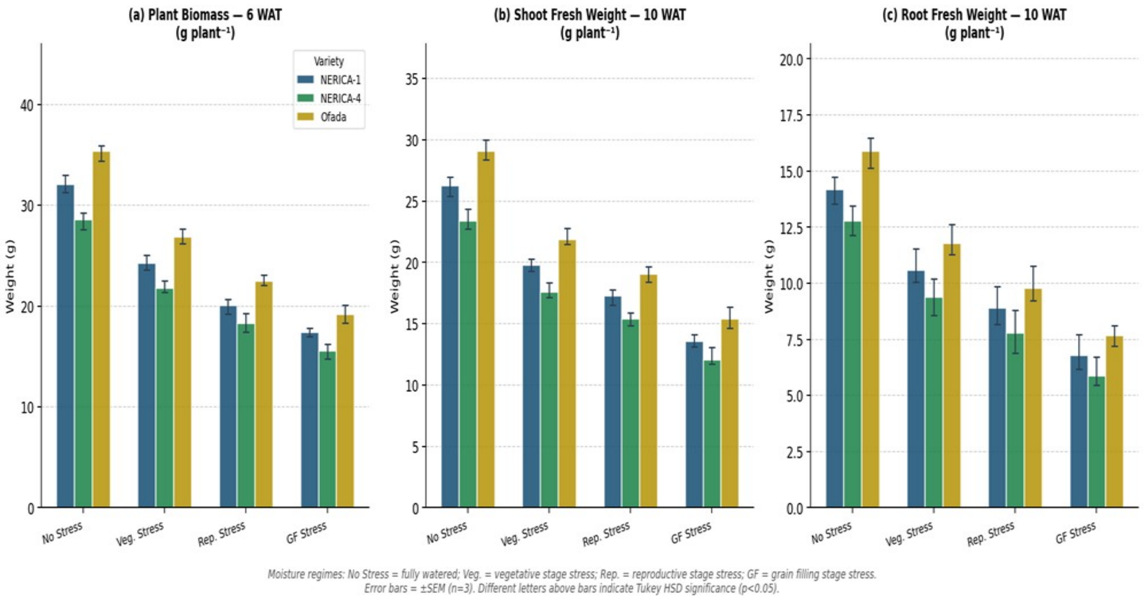

Fig. 6: (a) Plant biomass (g) at 6 WAT, (b) shoot fresh weight (g) at 10 WAT, and (c) root fresh weight (g) at 10 WAT across moisture regimes and rice varieties. Error bars = ±SEM (n=3). Moisture regimes: No Stress, Veg. = vegetative stress, Rep. = reproductive stress, GF = grain filling stress.

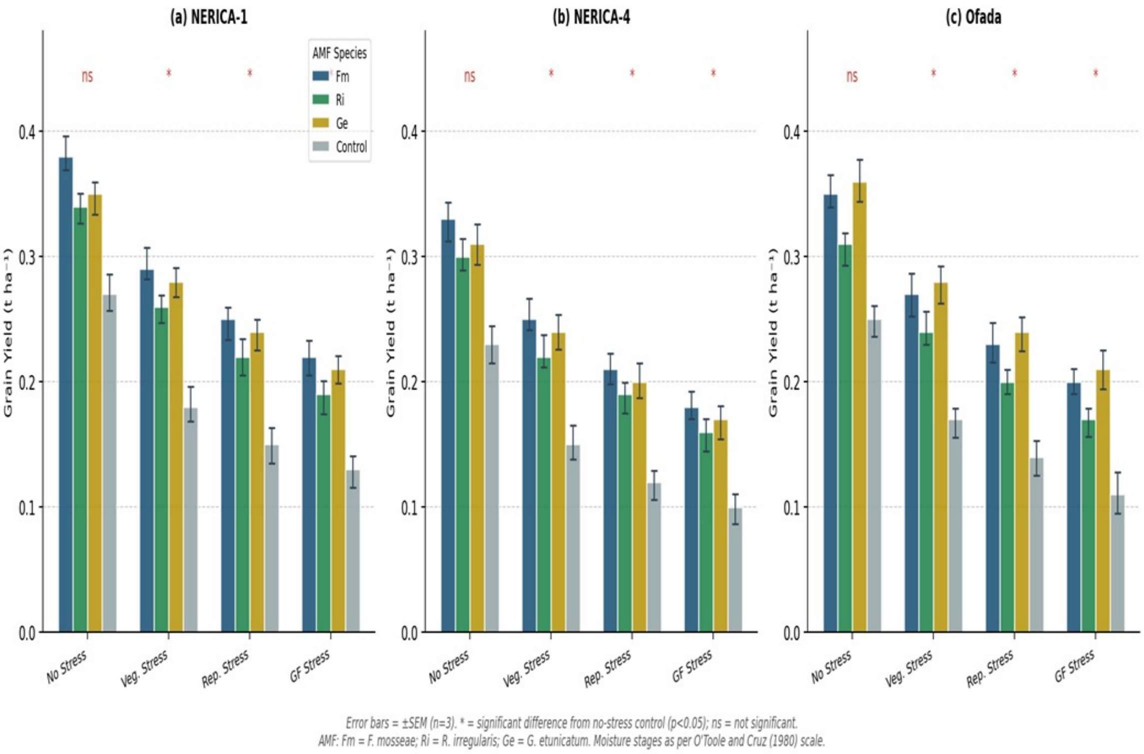

Fig. 7: Grain yield (t ha^{-1}) of: (a) NERICA-1, (b) NERICA-4 and (c) Ofada rice varieties under four AMF inoculation treatments and four moisture stress regimes. Error bars = ±SEM (n=3). * = significant difference from no-stress control at $p<0.05$; ns = not significant.

CONCLUSIONS

Arbuscular mycorrhiza inoculation significantly enhanced drought tolerance in rice varieties under stage-specific moisture stress.

Glomus etunicatum inoculation consistently produced the highest AMF root colonization (arbuscule, vesicle, and hyphae percentages) across all varieties and moisture regimes. *Funneliformis mosseae* significantly increased grain yield in NERICA-1 and N, P, K uptake in Ofada variety,

Moisture stress at the reproductive stage caused the greatest yield reduction, while AMF inoculation mitigated yield losses.

AMF-driven root and biomass improvements are the primary pathways to yield maintenance under drought.

Ge inoculation is the most effective species for root system enhancement.

It is therefore recommended that *Glomus etunicatum* or *Funneliformis mosseae* inoculation be adopted for upland rice production in southwestern Nigeria, particularly in rainfed cropping systems susceptible to seasonal drought.

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