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Genotype and Nitrogen Source Influence Drought Stress Response in Oil Palm Seedlings

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Abstract: As a significant global source of vegetable oil, the oil palm's ability to withstand abiotic stresses, particularly drought, is crucial for sustainable agriculture. This is especially significant in tropical regions, where water scarcity is becoming more common. Nitrogen, a vital nutrient, plays an essential role in various physiological and biochemical processes in plants, directly influencing growth and stress tolerance. This study investigates the interaction between nitrogen sources (ammonium vs. nitrate) and drought stress in oil palm (*Elaeis guineensis*) seedlings, which is critical in enhancing productivity in this economically important crop. The experiment evaluated five commercial oil palm genotypes, which were supplied with nitrogen solutions (15 mM NH_4^+ or NO_3^-) for 46 days, followed by 30 days of progressive drought. The results showed that drought conditions universally reduced the biomass, with ammonium-fed plants exhibiting greater shoot biomass sensitivity than nitrate-fed plants. Drought also significantly decreased the chlorophyll a, PhiPS2, and root-reducing sugar levels—critical indicators of photosynthetic efficiency and overall plant health. The effects on the root architecture were complex, with ammonium nutrition differentially influencing the lateral root length under well-watered versus drought conditions, highlighting nitrogen forms' nuanced role in root development. Importantly, substantial genotypic variability was observed in most traits, affecting the responses to both the nitrogen source and drought stress. This variability suggests that certain genotypes may be better suited to cultivation in specific environmental conditions, particularly drought-prone areas. In conclusion, this study underscores the intricate interplay between nitrogen nutrition, genotypic variability, and drought tolerance in oil palm seedlings. These findings highlight the need to integrate these factors into agricultural management strategies to improve resilience and productivity in oil palm plantations.

Keywords: ammonium; nitrate; water deficit; plant performance

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1. Introduction

In Colombia, oil palm cultivation is a key agricultural sector, spanning nearly 600,000 hectares and producing around 1.8 million tons in 2022. This positions oil palm as the second-largest crop in the country and the fourth-largest contributor to agricultural exports [1–3]. However, Colombia has been increasingly affected by the El Niño phenomenon, which has caused up to a 40% reduction in production in certain areas, particularly in the northern region [4]. El Niño is characterized by the extensive warming of the Pacific Ocean's surface off the coast of South America, often leading to reduced precipitation. In Colombia, this has led to lower water levels in rivers and other critical water sources for agriculture. Climate change is likely to exacerbate and prolong these events, further restricting the water availability and limiting both water access and nutrient uptake for crops.

Nitrogen is essential in boosting crop productivity, yet its availability is limited in many soils. Nitrogen is typically applied to crops as urea, nitrate, or ammonium to address this. However, the indiscriminate use of fertilizers has resulted in lower nitrogen use efficiency and increased nitrogen loss to the environment, leading to adverse effects such as eutrophication and increased greenhouse gases, including N_2O. Although ammonium is often considered toxic to most crops at lower concentrations than nitrate, our previous research has shown that oil palm plants demonstrate high tolerance to ammonium [5]. Consequently, ammonium may be a viable and environmentally friendly alternative to nitrate fertilization.

Despite these benefits, the effect of different nitrogen sources on oil palm's adaptation to drought stress still needs to be explored. Previous studies suggest that ammonium may be more beneficial in enhancing drought tolerance than nitrate [6]. Ammonium has been shown to improve plant drought resilience by promoting water uptake and modifying the root characteristics [7]. In contrast, nitrate assimilation requires significant energy expenditure, as plant cells must first convert nitrate into ammonium. During stress events like drought, plants may prioritize survival mechanisms over growth processes, potentially reducing the nitrogen use efficiency.

Moreover, the uptake of nitrate or ammonium can alter the rhizosphere's pH, which in turn influences the solubility and availability of other nutrients. Nitrate typically raises the pH levels, promoting the uptake of cations such as H^+, K^+, Na^+, Cd^{2+}, and Zn^{2+}, while inhibiting anion absorption, including Cl^- and ${SO_4}^{2-}$ [8]. This behavior is generally consistent in plants receiving nitrate nutrition, regardless of the stress conditions. On the other hand, ammonium uptake tends to lower the pH in the rhizosphere [9]. However, ammonium-related responses under stress conditions are not as well understood as those linked to nitrate. This gap in understanding may stem from ammonium's potential toxicity and lower uptake rate compared to nitrate. Therefore, further research is needed to clarify ammonium's role in plant responses to stress.

In this study, we selected five contrasting oil palm cultivars and exposed them to ammonium or nitrate before subjecting them to drought stress. The chosen nitrogen concentration of 15 mM was based on a prior study, which demonstrated that this concentration, regardless of the nitrogen source, had no adverse effect on plant growth. While growth was maintained with nitrate, it increased with ammonium in a dose-dependent manner [5]. Studies in cotton have indicated that elevated nitrogen levels can mitigate drought stress by enhancing the activity of nitrogen-metabolizing enzymes and increasing the levels of osmoprotectants, such as free amino acids and total soluble proteins [10]. Moreover, Marino [11] observed that while high ammonium concentrations often reduce plant growth, they can confer benefits by boosting the resistance to other stressors like drought, either by activating shared defense mechanisms or by triggering enhanced defense responses via nitrogen metabolism.

These findings prompted us to investigate how oil palm responds to high concentrations of these two nitrogen sources in combination with drought stress across different cultivars. We specifically evaluated the shoot and root biomass, root length, physiological parameters (gas exchange and chlorophyll fluorescence), and biochemical indices (chlorophyll a content, soluble proteins, free amino acids, reducing sugars, nitrogen, phosphorus, potassium calcium, magnesium and boron percentages in leaves). By examining these interactions, we aim to gain insights into potential strategies to improve the resilience of oil palm cultivation under increasingly challenging environmental conditions.

2. Materials and Methods

2.1. Growth Conditions and Experimental Design

The experiment was conducted in a mesh house with 40% polyshade cover. Five oil palm progenies were evaluated in this study: four *Elaeis guineensis* progenies and one interspecific hybrid (O × G). The *E. guineensis* progenies included two widely planted commercial cultivars, Deli × AVROS (D × A) and Deli × La Mé (D × LM). D × A is known for its high oil extraction rate (OER), high average bunch weight (ABW), and low bunch number (BN), while D × LM, originating from West Africa, exhibits a high BN, low ABW, and high OER. Additionally, two experimental progenies were tested: *dura* × *pisifera* big (D × P big), which has an annual growth rate of 0.6 m year^{-1}, and *dura* × *pisifera* small (D × P small), with a slower growth rate of 0.29 m year^{-1}. The interspecific hybrid O × G was the commercial cultivar Coari × La Mé, known for its high productivity and extraction rate.

The progenies were tested under two nitrogen sources—ammonium and nitrate—and two water conditions—well-watered and drought-stressed. Germinated seeds were transferred to 3 L containers filled with perlite and vermiculite. For the first eight weeks post-germination, the seedlings were irrigated with 50 mL of deionized water at regular intervals. At the eight-week mark, the endosperm was carefully separated from the seedling to eliminate external nutrient inputs, and the attached seed was excised. Nitrogen treatments were then initiated, with plants receiving three weekly applications (Mondays, Wednesdays, and Fridays) of 100 mL nutrient solution to maintain the field capacity. Nitrogen was provided in two forms: 7.5 mM calcium nitrate ($Ca(NO_3)_2$) to supply nitrate and 7.5 mM ammonium sulfate ($(NH_4)_2SO_4$) to supply ammonium. The nutrient solution was supplemented with a blend of macro- and micronutrients described by De la Peña et al. [12], and the pH was adjusted to 6.3. Plants receiving nitrate-based nutrition were supplemented with calcium sulfate to maintain consistent sulfate levels.

In week 15, water regulation was initiated based on the nitrogen treatment. The control group was regularly irrigated every two days with deionized water to maintain the soil field capacity (−0.05 MPa). In contrast, the drought group was subjected to water deprivation until the experiment concluded in week 19, which lasted 30 days without irrigation. The soil water potential, measured using a Theta Probe® (Delta Technologies, Cambridge, England), was around −1 MPa for all genotypes (Table 1). The experiment followed a factorial randomized complete block design with five replicates and three pots as the experimental unit. The experimental factors were the five progenies, two nitrogen sources, and two water conditions.

Table 1. Soil water potential (MPa) of five oil palm genotypes after 30 days of progressive drought under ammonium or nitrate nutrition.

Genotype	Ammonium (MPa)	Nitrate (MPa)
C × LM	−1.85 ± 0.5	−1.22 ± 0.56
D × A	−1.05 ± 0.27	−0.95 ± 0.48
D × LM	−0.95 ± 0.27	−0.98 ± 0.24
D × P (large)	−1.26 ± 0.37	−0.87 ± 0.34
D × P (small)	−1.16 ± 0.32	−0.95 ± 0.49

2.2. Biomass, Root Length, Physiological Parameters, and Biochemical Indexes

At week 19, after the onset of drought conditions, we performed gas exchange measurements, including the net photosynthetic rate (An), stomatal conductance (gs), intercellular CO_2 (Ci), and transpiration rate (E), along with fluorescence parameters such as the quantum yield of photosystem II (PhiPS2) and nonphotochemical quenching (NPQ). These measurements were taken on the first fully expanded leaf. Leaf acclimation measurements in the dark were conducted between 8:00 p.m. and 9:30 p.m. In contrast, gas exchange measurements were performed between 8:30 a.m. and noon using a portable infrared gas analyzer (IRGA, model LI-6800, LI-COR Inc., Lincoln, NE, USA) under a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1}. The airflow rate into the chamber was set to 300 μmol s^{-1}, the leaf temperature was maintained at 28 °C, the relative humidity at 60%, and the chamber CO_2 concentration at 400 μmol mol^{-1}. Chlorophyll fluorescence measurements were carried out using 1000-ms-long rectangular flashes of 10,000 μmol photon m^{-2} s^{-1} intensity with a 250 kHz flash modulation rate. In the dark, the fluorescence was measured using 500 Hz modulation, while, in the light, it was measured with 50 kHz modulation.

At the end of the experiment, fresh shoot and root matter were collected and weighed. The lengths of the primary root (PR) and lateral roots (LR) were analyzed using the RhizoVision Explorer Version 2.0.2 software, with roots categorized based on diameter: lateral roots had a diameter < 1.5 mm, and primary roots had a diameter > 1.5 mm. The plant fractions were oven-dried at 80 °C for 48 h to estimate the dry matter content or frozen in liquid nitrogen and stored at −80 °C for subsequent biochemical analyses. The concentrations of key metabolites—including total chlorophyll, proteins, amino acids, and reducing sugars—were determined using colorimetric methods in both roots and leaves. Additionally, the levels of critical elements such as total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and boron (B) were quantified.

Chlorophylls and amino acids were extracted using an ethanol-based method [13], and amino acids were quantified using a ninhydrin-based method [14]. After ethanolic extraction, the pellet was resuspended in NaOH for protein content analysis using the Bradford assay [15]. The reducing sugar concentration was measured using the Nelson–Somogyi method [16]. For leaf element content analysis, dried samples were processed at the Leaf and Soil Analysis Laboratory at Cenipalma (LAFS), where wet acid digestion was used to extract elemental constituents. Nitrogen (N) and phosphorus (P) content was determined via colorimetric methods, while potassium (K), calcium (Ca), and magnesium (Mg) content was measured using atomic absorption spectroscopy. The azomethine-H method was used to assess the boron (B) content through colorimetry [17].

2.3. Statistical Analysis

The data were analyzed using a factorial ANOVA with a linear model. Type III sum of squares was applied for significant interactions, and Type II sum of squares was used for non-significant interactions. The analysis was conducted using the “stats” and “car” packages in R version 4.3.1 [18]. Before the analysis, the assumptions of normality and homogeneity of variance were tested to ensure the model’s validity.

3. Results

3.1. Growth and Development

This study investigated the effects of nitrogen sources on drought stress in five commercial oil palm genotypes. Plants were supplied with 15 mM NH_4^+ or NO_3^- for 46 days, followed by 30 days of progressive drought. Despite the potentially harmful effects of high ammonium concentrations and drought on plants, no visible stress symptoms were observed in the oil palm plants at harvest. The results indicated that nitrogen treatment significantly influenced the drought response in the shoot biomass ($p < 0.01$). Although drought stress reduced the shoot biomass, the decrease was more pronounced in ammonium-fed plants. Additionally, drought significantly reduced the root biomass, underscoring the critical role of water availability in root development. These effects were observed across all genotypes, although the magnitude varied significantly between genotypes ($p < 0.001$) (Figure 1).

The effect of the nitrogen source on the root-to-shoot ratio varied significantly among the genotypes ($p < 0.01$). Most genotypes displayed a higher root-to-shoot ratio in ammonium-fed plants, except D × LM (Figure 2).

The nitrogen source slightly impacted the total root length and lateral root length under drought conditions (Figure 3A,B), with nitrate-fed plants showing a somewhat more significant reduction than ammonium-fed plants. However, neither the nitrogen source nor drought conditions affected the primary root length, although the length varied significantly among the genotypes ($p < 0.01$) (Figure 3C).

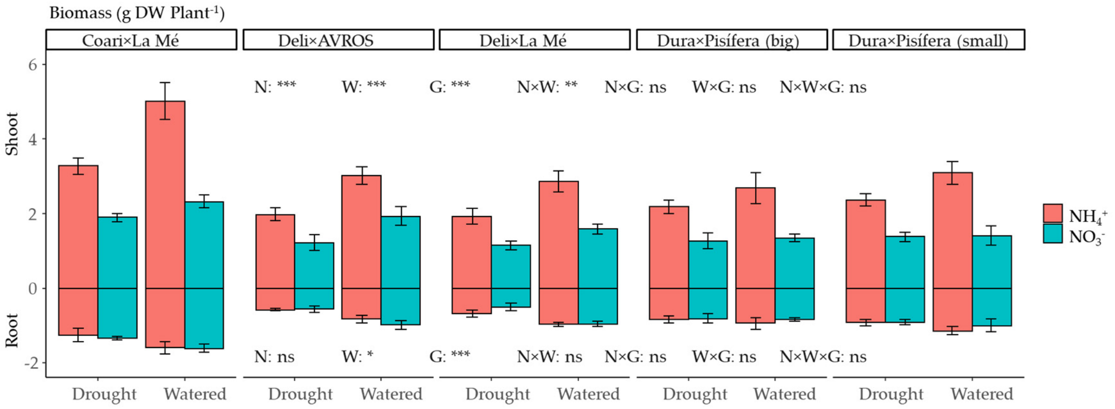

Figure 1. Average dry weight of the shoots and roots of five oil palm genotypes across two different nitrogen forms (ammonium and nitrate) and two water conditions (drought and watering). Statistical significance levels for the main effects and interactions among N sources (N), water availability (W), and genotypes (G) are denoted by asterisks (* <0.05, ** <0.01, *** <0.001), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

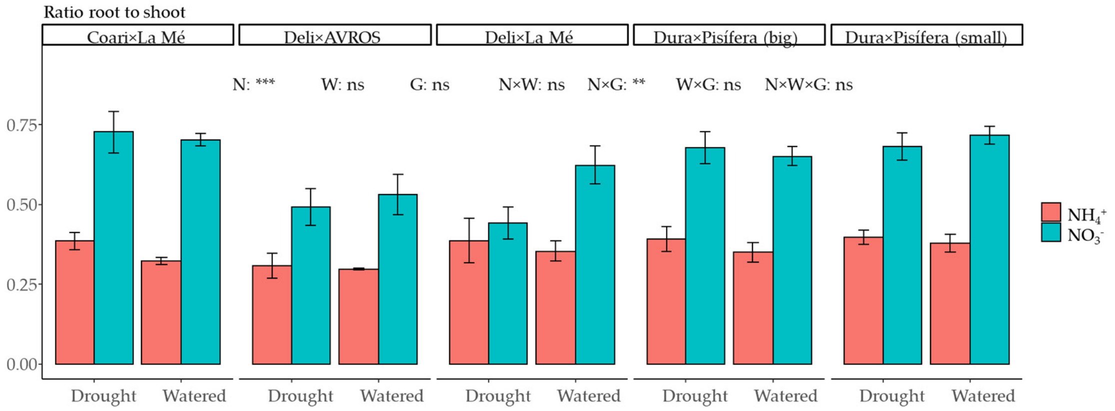

Figure 2. Root-to-shoot ratio for each genotype across two nitrogen forms (ammonium and nitrate) and two water conditions (drought and watered). Statistical significance levels for the main effects and interactions among N sources (N), water conditions (W), and genotypes (G) are denoted by asterisks (** <0.01, *** <0.001), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

3.2. Leaf Gas Exchange and Fluorescence Parameters

Drought stress significantly decreased gs, Ci, and PhiPS2 (p < 0.05, 0.01, or 0.001), regardless of the genotype or nitrogen source (Figurs 4B,C and 5A). While An was reduced under drought conditions, it was influenced by the genotype, suggesting genetic variability (Figure 4A). Genotype-specific responses were particularly noticeable in E under the different nitrogen forms and water conditions (Figure 4D). Interestingly, the nitrate source increased NPQ (p < 0.05) across all genotypes, with the absolute NPQ values varying depending on the genotype.

3.3. Metabolite Content

The chlorophyll a content in the leaves decreased under drought conditions (Figure 6A). The protein content and reducing sugar levels responded to the nitrogen source, although this response varied by genotype (p < 0.05) (Figure 6B,D). The amino acid content was influenced by nitrogen nutrition but was also significantly affected by both the water conditions and genotype (p < 0.001) (Figure 6C).

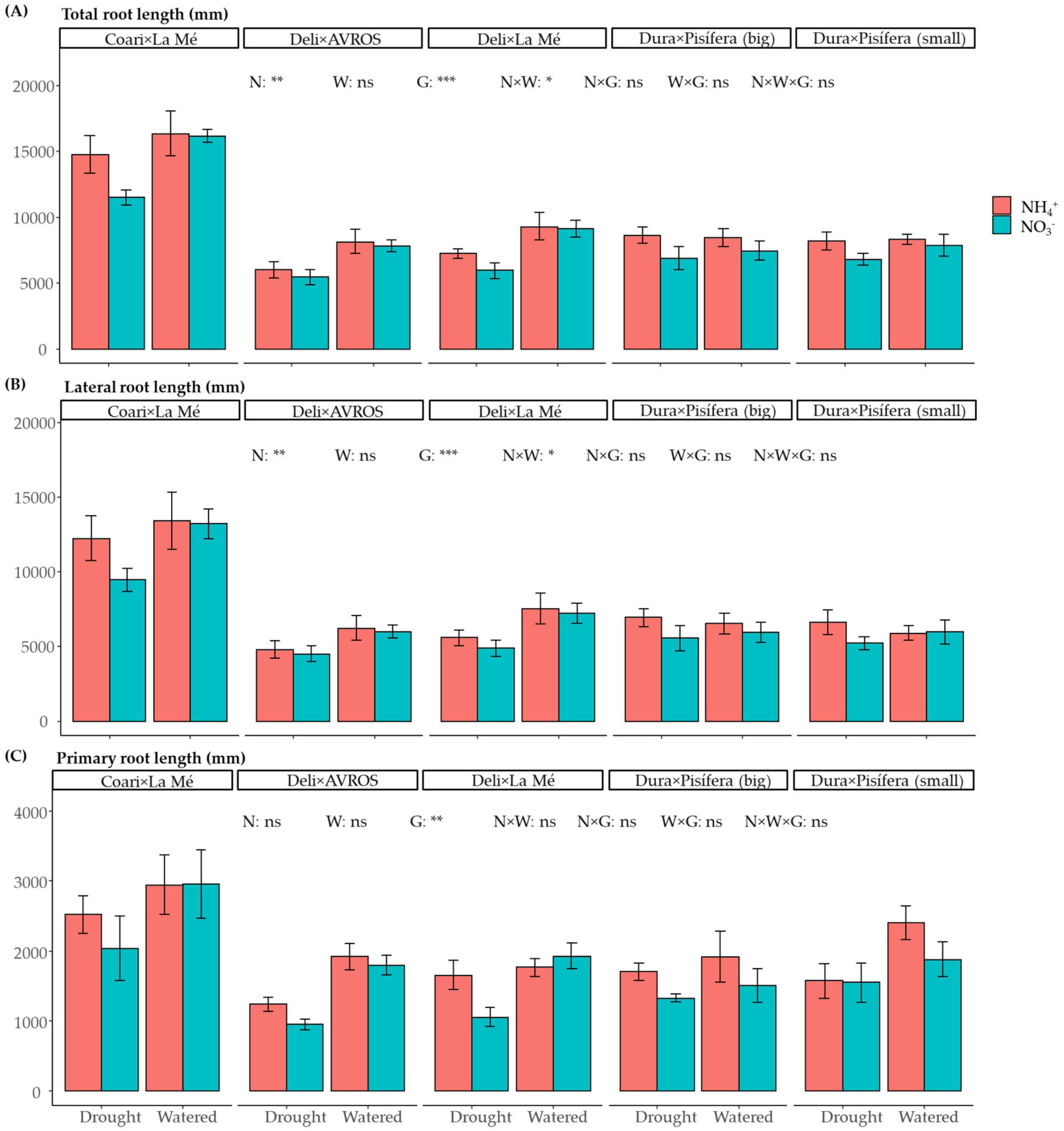

Figure 3. The graph displays the average (**A**) total, (**B**) lateral, and (**C**) primary root length for each genotype across two different nitrogen forms (ammonium and nitrate) and two water conditions (drought and watering). Statistical significance levels for the main effects and interactions among N sources (N), water conditions (W), and genotypes (G) are denoted by asterisks (* <0.05, ** <0.01, *** <0.001), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

Drought stress and the nitrogen source were the main factors affecting the reducing sugar content in the roots. Ammonium-fed plants exhibited higher levels of reducing sugars than nitrate-fed plants, and drought stress generally increased the reducing sugar content (Figure 7C). The protein content, however, was not significantly influenced by any of the factors studied ($p < 0.001$) (Figure 7A). The root amino acid content was affected by the nitrogen source, although this effect varied by genotype (Figure 7B).

Figure 4. Gas exchange for each genotype across two different nitrogen forms (ammonium and nitrate) and two water conditions (drought and watered) after the imposition of water conditions: (**A**) net photosynthesis (A_n), (**B**) stomatal conductance (g_s), (**C**) substomatal CO_2 concentration (C_i), and (**D**) transpiration rate (E). Statistical significance levels for the main effects and interactions among N sources (N), water conditions (W), and genotypes (G) are denoted by asterisks (* <0.05, *** <0.001), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

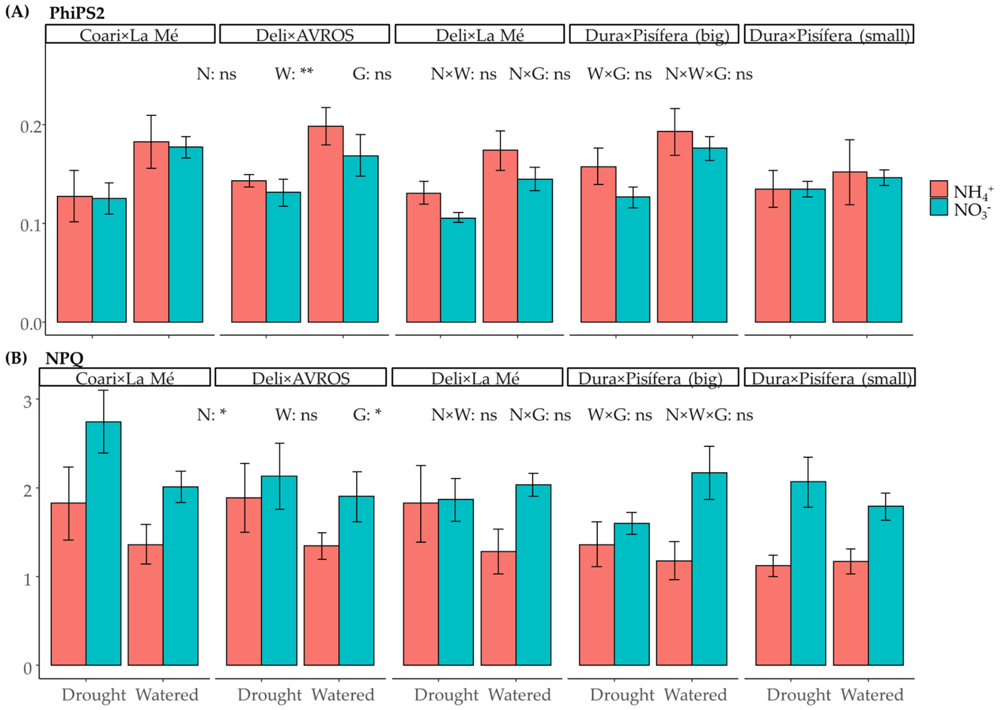

Figure 5. Chlorophyll a fluorescence across two different nitrogen forms (ammonium and nitrate) and two water conditions (drought and watered) after the imposition of water conditions: (**A**) quantum yield of photosystem II (PhiPS2) and (**B**) nonphotochemical quenching (NPQ). Statistical significance levels for the main effects and interactions among N sources (N), water conditions (W), and genotypes (G) are denoted by asterisks (* <0.05, ** <0.01), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

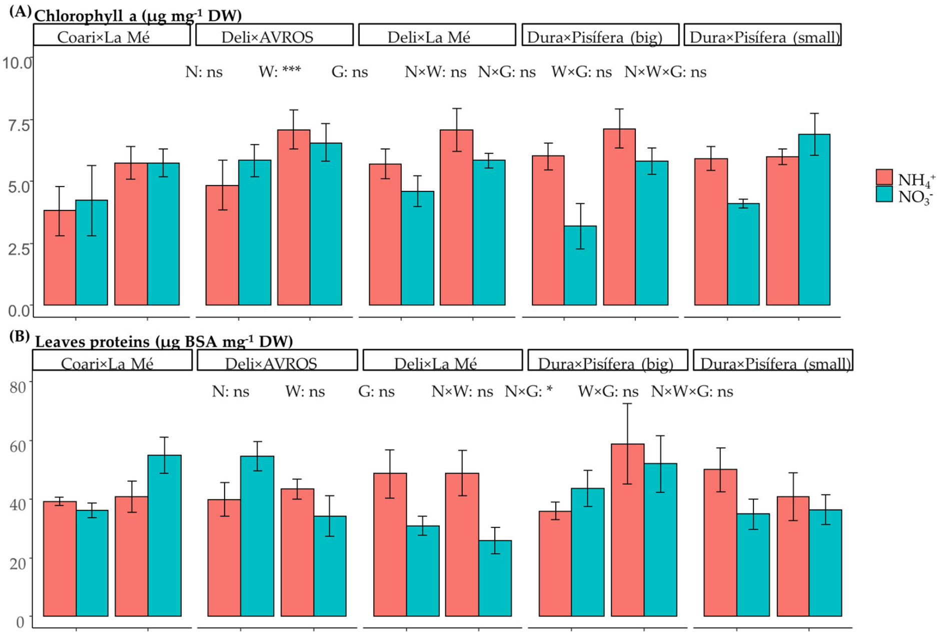

Figure 6. *Cont.*

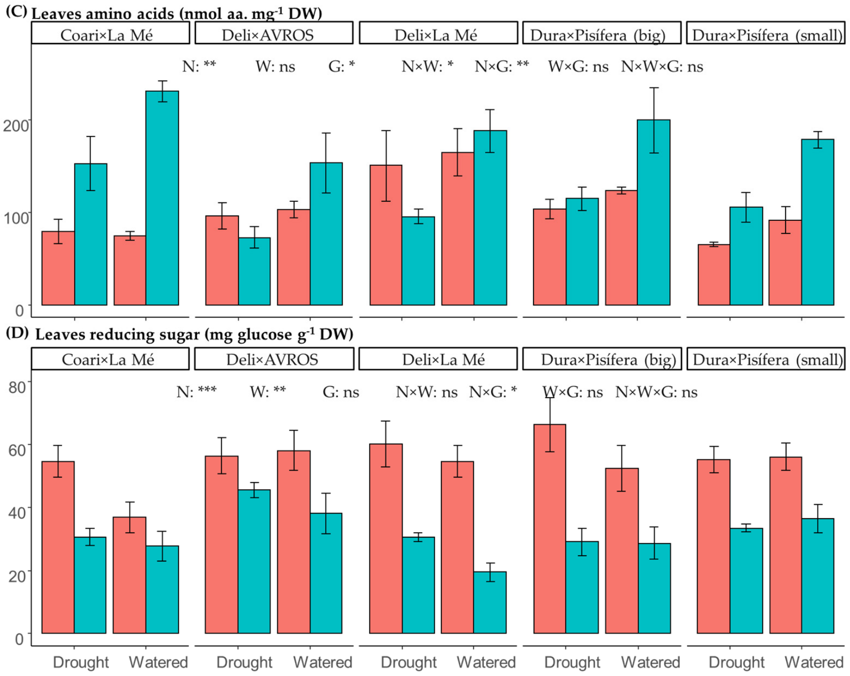

Figure 6. Leaf metabolic content across two different nitrogen forms (ammonium and nitrate) and two water conditions (drought and watered) after the imposition of water conditions: (**A**) chlorophyll a, (**B**) proteins, (**C**) amino acids, and (**D**) reducing sugars for each genotype. Statistical significance levels for the main effects and interactions among N sources (N), water conditions (W), and genotypes (G) are denoted by asterisks (* <0.05, ** <0.01, *** <0.001), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

Figure 7. Root metabolic content across two different nitrogen forms (ammonium and nitrate) and two water conditions (drought and watered) after the imposition of water conditions: (**A**) proteins,

(**B**) amino acids, and (**C**) reducing sugars for each genotype. Statistical significance levels for the main effects and interactions among N sources (N), water conditions (W), and genotypes (G) are denoted by asterisks (* <0.05, ** <0.01, *** <0.001), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

3.4. Foliar Content of Essential Minerals

The percentage of nitrogen was significantly influenced by the interaction between the nitrogen source and genotype ($p < 0.01$) (Figure 8A). Drought stress significantly affected the phosphorus levels, with this impact varying based on the nitrogen source and genotype (Figure 8B). The effects of the nitrogen sources on the phosphorus levels also depended on the genotype. The responses of the potassium and boron percentages to drought stress varied according to the nitrogen source (Figure 8C). In contrast, the calcium percentage responses to drought stress were influenced by both the nitrogen source and genotype (Figure 8D). The magnesium percentage was affected by the nitrogen source, although this effect varied with the genotype (Figure 8E).

Figure 8. *Cont.*

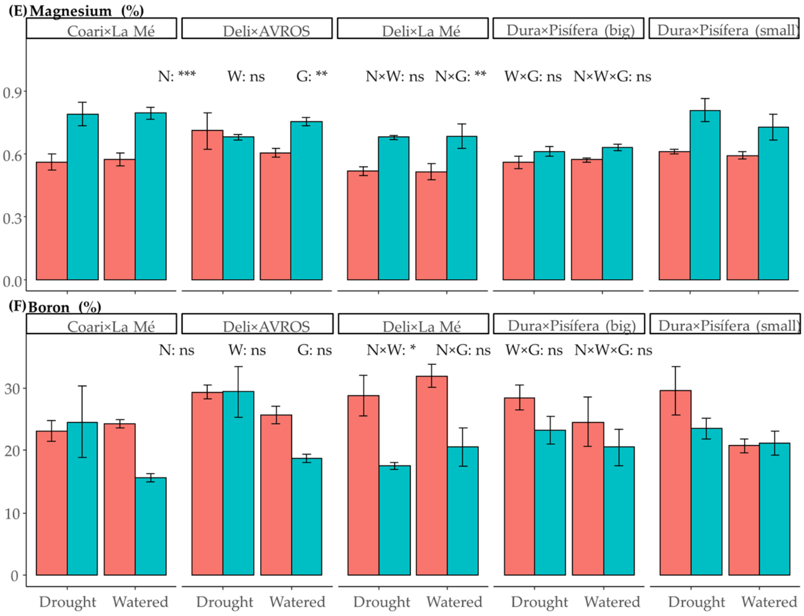

Figure 8. Leaf mineral content across two different nitrogen forms (ammonium and nitrate) and two water conditions (drought and watered) after the imposition of water conditions: (**A**) nitrogen, (**B**) phosphorus, (**C**) potassium, (**D**) calcium, (**E**) magnesium, and (**F**) boron for each genotype. Statistical significance levels for the main effects and interactions among N sources (N), water conditions (W), and genotypes (G) are denoted by asterisks (* <0.05, ** <0.01, *** <0.001), and nonsignificant results are labeled ns. Error bars represent standard errors (n = 5).

4. Discussion

Ammonium and nitrate are plants' primary nitrogen sources, but, when supplied as the sole nitrogen source, ammonium is typically toxic at elevated concentrations for most crops. Contrary to expectations, our previous study showed that oil palm plants preferred higher ammonium concentrations over nitrate under well-watered conditions [5]. However, the nitrogen source preference can vary depending on the species, plant growth stage, and environmental conditions [19]. To evaluate the effects of nitrogen sources on drought tolerance in this study, we deprived five oil palm genotypes of water for 30 days after exposure to ammonium or nitrate. Other studies have subjected young oil palms to progressive drought for various durations, such as 14 days [20,21], 24 days [22], 28 days [23], 40 days [22], and 57 days [24]. The variation in the drought exposure period can be attributed to differences in the substrate, nutrient levels, container size, plant growth stage, or desired stress level.

Our previous research demonstrated that ammonium-fed oil palm seedlings developed greater shoot biomass than nitrate-fed ones under sustained application [5]. In this study, we compared the biomass of drought-stressed plants with that of a control group at harvest. Both groups received equal nitrogen quantities by the time of harvest, and no additional nutrients were supplied during the final 30 days. The results showed that the ammonium-fed plants exhibited a more significant reduction in the shoot biomass growth rate under drought conditions than the nitrate-fed plants. This suggests that the initial advantage in shoot biomass from ammonium nutrition may paradoxically reduce the drought resilience, possibly due to increased transpiration from a larger leaf area [25]. However, one limitation of this study is the need for morphological measurements, particularly the leaf area, which could have provided more insight into the relationship between biomass and drought tolerance. An alternative explanation is that the ammonium-fed control group experienced a higher growth rate during the stress period than the nitrate-fed group. Future studies should harvest a subset of plants before initiating drought stress. Comparing these pre-drought results with those from the end of the experiment would help to clarify whether the ammonium-fed plants had a higher growth rate and explain the more pronounced differences in the drought response between the two nitrogen sources.

Biomass partitioning between the shoot and root is critical in maximizing growth under stress conditions. Our findings indicate that the nitrogen source affects this partitioning, with the nitrate-fed plants generally showing a higher root-to-shoot ratio than the ammonium-fed plants. However, this difference was also influenced by the genotype. This trend seems to stem more from the inhibition of the shoot biomass under nitrate nutrition, rather than an increase in root growth. This conclusion is supported by our observation that the root biomass was similar in the nitrate- and ammonium-fed plants, whereas the ammonium-fed plants developed greater shoot biomass. A similar result was found in pea plants, where nitrate-fed plants were compared to nitrogen-fixing plants [26].

We further investigated the root system architecture. Under drought stress, the reduction in the total and lateral root lengths was influenced by the nitrogen sources, with nitrate-fed plants showing a slightly more pronounced reduction compared to ammonium-fed plants. However, neither the nitrogen source nor water availability significantly affected the primary root length. Similar outcomes were reported by Ma et al. [19] in *Erythropalum* sp., a species that favors ammonium over nitrate. In that case, no significant differences were detected in the primary root length, although differences were observed in the fibrous root dry weight. We hypothesize that oil palm seedlings may prioritize maintaining lateral and total root growth under ammonium nutrition due to its lower assimilation energy requirements than nitrate.

Given that nitrogen regulates many processes of photosynthesis [27], we investigated the effect of nitrogen nutrition on photosynthesis and chlorophyll fluorescence under drought stress. The significant reduction in the net photosynthetic rate (An) under drought conditions was genotype-dependent but not influenced by the nitrogen sources. The stomatal conductance (gs), intercellular CO_2 concentration (Ci), and PhiPS2 were reduced under drought stress across all nitrogen sources and genotypes. However, the nitrogen source significantly influenced nonphotochemical quenching (NPQ) and the transpiration rate (E), with the effect on E further modulated by the water availability and genotype. These results are consistent with our previous findings under well-watered conditions [5], although they contrast those of some studies where the nitrogen forms differently affected the gas exchange parameters. For instance, ammonium has been shown to enhance the photosynthetic capacity in *Cunninghamia lanceolata* [19] and reduce leaf transpiration in tobacco plants [28]. In our study, the nitrogen effect on gas exchange was minor, with only specific genotypes (D×A, D×LM, and D×P) showing a slight tendency for ammonium-fed plants to have higher photosynthetic rates than nitrate-fed plants under well-watered conditions. However, the complex relationship between the genotype and water conditions may obscure the effects of the nitrogen sources on photosynthesis and other gas exchange parameters. Future experiments should address these complexities by increasing the number of replicates or including a broader range of genotypes to boost the statistical power.

Still, in this study, NPQ was significantly affected by the nitrogen source. It is plausible that nitrate nutrition increased the reducing power in plants, leading to greater thermal dissipation, as reflected in the increased NPQ. Interestingly, the highest NPQ was observed in the C×LM genotype under nitrate nutrition, suggesting that the C×LM hybrid may not transfer energy as efficiently as *E. guineensis*. In contrast, ammonium nutrition alleviated the heat dissipation (NPQ) reduction by stimulating the energy demand for CO_2 fixation during ammonium assimilation. However, Alencar et al. [29] found that toxic levels of NH_4^+, when combined with excess light, could disturb the photosynthetic apparatus of rice plants, resulting in higher NPQ. In our study, ammonium was not toxic, but further research is needed to explore the effects of toxic ammonium concentrations on photosynthetic parameters such as NPQ and PhiPS2 and to investigate the biochemical and physiological mechanisms behind these responses.

The chlorophyll a concentration decreased under drought stress, which correlated with the reductions in the photosynthetic parameters. This result aligns with previous studies showing that drought stress typically leads to lower chlorophyll content than in well-watered plants [30,31].

Osmoprotective solutes commonly accumulate under drought stress to prevent water loss. In this study, drought stress and nitrogen nutrition did not affect the root protein content. However, nitrogen nutrition significantly influenced the leaf protein content, although no consistent pattern emerged due to genotype-specific effects. Similarly, nitrogen nutrition affected the root amino acid concentrations and leaf-reducing sugar content, with a tendency for higher values under ammonium nutrition, although these effects also varied by genotype. In contrast, the leaf amino acid concentrations were influenced by the nitrogen sources, showing a trend toward higher levels of nitrate than ammonium, albeit again varying by genotype. Drought stress also affected the leaf amino acid concentrations depending on the nitrogen source. The root-reducing sugar content was influenced by drought and the nitrogen source, with a tendency to increase under drought stress and be higher in ammonium-fed plants than in nitrate-fed plants.

These findings suggest that nitrogen sources and drought stress have distinct effects on solutes in the leaves and roots of oil palm plants, with variations largely dependent on the genotype. Previous studies have reported that plants fed ammonium at toxic concentrations show a marked difference in amino acid accumulation between the roots and leaves, with greater accumulation in the roots [32]. This occurs because assimilation primarily takes place in the roots to prevent ammonium from reaching the leaves, where it could damage the photosynthetic apparatus [33]. However, in this study, ammonium nutrition did not show the clear accumulation pattern observed previously. This discrepancy could be due to the experimental design, as the plants were maintained for 30 days without a nutrient solution, allowing them to reutilize nitrogen to produce proteins or sugars, which could have been translocated to the roots. Additionally, nitrate assimilation primarily occurs in the leaves, as its reduction requires energy directly derived from photosynthesis [34]. This may explain why the nitrate-fed plants exhibited slightly higher amino acid accumulation in the leaves than the ammonium-fed plants, although this effect was genotype-dependent.

Water scarcity and nutrient uptake are intricately linked, as plants rely on dissolved nutrients in water for growth. Nitrogen is one of the most critical nutrients in enhancing productivity, yet its availability is limited in many soils. This limitation becomes even more pronounced under water-limited conditions. In our study, the leaf nitrogen content was reduced under drought stress, but the effect of the nitrogen source varied by genotype. Moreover, nitrogen uptake, whether in the form of ammonium or nitrate, is closely tied to the absorption of other nutrients, as ions compete for transport across cell membranes.

Ammonium nutrition generally negatively affects the osmotic regulation of leaves due to the reduced absorption of cations like K^+, Mg^{2+}, and Ca^{2+} [35]. In this study, the nitrogen source affected the potassium content, although this effect was influenced by the water availability. For instance, the potassium content slightly increased under drought conditions with ammonium nutrition. The magnesium content decreased under ammonium nutrition, but this effect was genotype-dependent. Calcium was also reduced under ammonium nutrition, but this effect depended on the water conditions and genotype. Despite this reduction, the calcium levels remained above the 0.3% threshold, considered deficient for oil palm [35].

Additionally, ammonium nutrition resulted in higher phosphorus uptake, a response also observed in rice [36]. However, this increase varied in magnitude depending on the water availability and genotype. Drought stress also affected the phosphorus concentration, with the genotype playing a key role in this variation. The drought conditions influenced the boron levels, but no clear pattern emerged due to the dependence on nitrogen sources. In cases where the boron concentration was higher under drought conditions, such as in ammonium-fed plants of the D×LM genotype, this could be advantageous for aquaporin activity and improved hydraulic conductance [21].

Overall, the effect of the nitrogen sources on drought stress was highly complex. Many traits, including the root-to-shoot ratio, net photosynthesis (An), transpiration rate (E), leaf biochemical composition (proteins, amino acids, reducing sugars), root amino acids, and leaf nutrient percentages (nitrogen, phosphorus, calcium, and magnesium), were influenced by both environmental factors and the genotype. Notably, the interactions between these factors, especially those involving the genotype, hold significant value for breeding programs. This genetic diversity enables the selection and breeding of plants with enhanced performance under specific conditions. In contrast, traits such as the shoot biomass, root biomass, total lateral and primary root lengths, and nonphotochemical quenching (NPQ) showed consistent genotype-specific effects. While these traits demonstrated genetic differences, they exhibited uniform responses to drought stress or nitrogen source variations. This complexity offers valuable insights for crop improvement strategies, allowing breeders to focus on traits with diverse genetic responses for adaptation to specific environments while capitalizing on other traits' stability across different conditions. Ultimately, this knowledge can guide the development of oil palm varieties with improved drought tolerance and nitrogen use efficiency, addressing critical agricultural challenges in a changing climate.

5. Conclusions

This study provides insights into the complex interactions between nitrogen sources, drought stress, and genotypic variation in oil palm seedlings. Our findings lead to several important conclusions.

1. The choice of nitrogen fertilizer (ammonium vs. nitrate) can significantly impact the drought tolerance of oil palm seedlings, with potential long-term effects on plantation productivity under water-limited conditions.
2. The observed genotypic variations suggest that the careful selection of oil palm cultivars could simultaneously optimize the nitrogen use efficiency and drought tolerance.
3. The complex interactions between nitrogen nutrition and the root architecture under drought stress highlight the need for holistic approaches in breeding programs considering above-ground and below-ground plant responses.

Future research should focus on validating these findings in mature oil palm trees and under field conditions and exploring the molecular mechanisms underlying the observed genotype-specific responses to the nitrogen form and drought stress.

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