

Research article.

Influence of potassium nutrition on photosynthetic performance and antioxidant activity in cacao (*Theobroma cacao* L.) clones: Evidence from controlled-environment trials.

Jose Julian Apraez Muñoz¹; Álvaro Javier Ceballos Freire²; William Ballesteros Possu³; Jesús Giovanni Solarte⁴; Diego Javier Perez Ortega⁵;

¹PhD in Genetics and Molecular Biology. Professor, University of Nariño. Pasto, Colombia*.

²PhD in Agricultural Sciences. Professor, University of Nariño. Pasto, Colombia.

³PhD in Natural Resources Science. Professor, University of Nariño. Pasto, Colombia.

⁴PhD in Agricultural Sciences. Professor, University of Nariño. Pasto, Colombia.

⁵ PhD in Environmental Engineering UNESP. Professor, University of Nariño. Pasto, Colombia.

Abstract

Cacao (*Theobroma cacao*) cultivation is a strategic activity for rural development in Nariño, Colombia, offering sustainable livelihood opportunities in regions affected by social conflict and agroecological constraints. However, climate change and soil nutrient depletion increasingly challenge crop productivity and bean quality. This study aimed to evaluate the physiological and biochemical responses of the CCN51 cacao clone under controlled environmental conditions and different potassium (K) fertilization levels, to better understand adaptive mechanisms relevant to crop management in vulnerable systems. The trial was conducted in a greenhouse over six months, applying potassium at concentrations of 200 and 400 mg/kg. Photosynthetic performance was monitored using the Multispec 2.0 device, and biochemical responses were assessed through the activity of antioxidant enzymes, including glutathione peroxidase and ascorbate peroxidase. Results indicated that higher potassium doses enhanced biomass accumulation and maintained photosynthetic efficiency, while intermediate doses activated compensatory mechanisms that supported plant development. Furthermore, potassium application positively regulated antioxidant enzymatic activity, suggesting its essential role in physiological stability and redox balance. These findings provide valuable insights for improving cacao productivity and resilience through targeted fertilization strategies adapted to the specific agroecological conditions of regions such as Nariño.

Keywords: antioxidant metabolism; drought stress; mineral nutrition; photosynthesis; resilience.

1. Introduction.

Cocoa (*Theobroma cacao*) is a key export crop with high relevance for several departments in Colombia. This study acknowledges the challenges faced by cocoa cultivation due to drought and climate change, which significantly affect both yield and quality. In this context, water emerges as a strategic and vital resource for the economic development of agricultural regions (MADR, 2022). Globally, cocoa has been recognized for its economic and social importance, particularly in developing countries, where it constitutes a major source of income for thousands of rural families. Colombia stands out as one of the leading cocoa producers in Latin America, making a notable contribution to the national economy and rural employment. Within the country, the department of Nariño has emerged as a region with substantial potential for cocoa cultivation, due to its favorable agroecological conditions (Gobernación de Nariño, 2022, p.3).

However, cocoa production has been increasingly threatened by the frequency and intensity of drought events and the broader impacts of climate change. These phenomena can disrupt rainfall patterns and raise temperatures, negatively affecting plant development and fruit quality. Water stress, in particular, is a limiting factor for cocoa growth and productivity, as water is essential for key physiological processes such as photosynthesis, transpiration, and nutrient transport (Taiz et al., 2022, p.3).

Photosynthesis—the process by which plants convert solar energy into chemical energy—is fundamental for biomass production and crop yield (Jaimez et al., 2022). In cocoa, efficient photosynthesis ensures proper flower and fruit development, resulting in higher yields and improved quality. Therefore, understanding how water stress and climate change influence photosynthesis in cocoa is critical for designing management strategies aimed at mitigating these impacts and ensuring the sustainability of cocoa cultivation at global, national, and regional levels (Jaimez et al., 2022, p.2).

Water uptake by roots is a key process in maintaining plant hydration and enabling proper physiological functioning (Jaimez et al., 2022). Drought conditions can impair the roots' ability to extract water from the soil, thereby compromising photosynthesis and other metabolic functions (Kramer, 2022). Hence, studying the relationship between water stress, water absorption, and photosynthesis in cocoa clones cultivated in the municipality of Chachagüí, Nariño, can provide

valuable insights for the development of water-efficient management practices and enhance the crop's resilience under drought stress (Jaimez et al., 2022, p.2).

2. Materials and Methods

2.1. Study Site

The study was conducted in the village of Matarredonda, located two kilometers from the urban center of the municipality of Chachagüí, Nariño Department, Colombia. The geographic coordinates are between 1°11'60"N and 3°11'24"N latitude, and 78°32'60"W and 76°28'48"W longitude. The area has a mean annual temperature of 20°C and is situated at an altitude of 1980 meters above sea level (IDEAM, 2013). The experiment was carried out in a polyethylene-covered greenhouse, which allowed for the control of key environmental variables such as relative humidity and temperature, thereby minimizing external interference in the evaluated treatments.

2.2. Plant Material and Establishment

The plant material used in the experiment consisted of cocoa cuttings obtained from mother plants aged between five and ten years. The cuttings were planted in 15 kg plastic pots filled with a pre-conditioned substrate composed of soil and organic matter mixed at a 3:1 ratio (v/v).

The control treatment received complete fertilization, while the remaining treatments were supplemented monthly with varying doses of potassium, applied as potassium chloride (KCl), according to the experimental design (Table 1). These applications aimed to reach a base saturation level of approximately 80%, an optimal condition for enhancing the availability of essential nutrients in the soil. The cuttings were then transplanted under uniform establishment conditions.

Throughout the experimental period, all treatments received the same amount of water to ensure consistent soil moisture levels, allowing for the observed responses to be attributed solely to the fertilization treatments. Physiological, photosynthetic, and biochemical variables were monitored continuously over a six-month period, following the successful establishment and rooting of the plants in the field.

Table 1. Description of Treatments

Treatments	Potassium Doses	Water ml
TO	Control treatment.	
T1	10 gr de KCl	100 ml.
T2	20 gr de KCl	
T3	30 gr de KCl	

2.4. Agronomic Variables

The morpho-agronomic variables evaluated included leaf size and shape, plant height, stem diameter, and total number of leaves. Leaf morphology was recorded through direct observation, considering potential alterations due to water stress, such as reduced surface area and changes in lamina shape. Plant height was measured using a measuring tape, from the base of the stem to the petiole of the last fully developed apical leaf. Stem diameter was measured 10 cm above the base of each plant using a digital caliper. The number of fully expanded leaves was determined by manual counting per individual in each experimental unit. These variables allowed for the identification of growth patterns associated with water stress and potassium fertilization treatments, serving as indicators of the structural performance and adaptive responses of the evaluated clones.

2.5. Photosynthetic Variables

Photosynthetic variables were measured using a portable MultiSpeQ v2.0 device (USA), equipped with a pulse-amplitude modulated (PAM) light system. The device was connected to the PhotosynQ platform (<https://photosynq.org>), using the “Photosynthesis RIDES no open/close” protocol as described by Kuhlger et al. (2016). The following parameters were recorded:

- **Maximum quantum efficiency of PSII (Fv/Fm):** Indicates the maximum proportion of light energy absorbed by PSII that is used in photochemistry, i.e., in converting light energy into chemical energy.

- **Effective quantum yield of PSII (Φ_{II}):** Reflects the operational photosynthetic efficiency of PSII under actual environmental conditions.
- **Linear electron flow (LEF):** Represents the process beginning with light absorption by PSII, followed by electron transport through a chain of carriers to PSI and ultimately leading to NADPH production.
- **Non-regulated energy dissipation (Φ_{NO}):** Estimated by applying far-red light for 30 seconds after the dark adaptation period, following Carstensen et al. (2018).
- **Non-photochemical quenching (NPQ):** A key mechanism used by plants to dissipate excess absorbed light energy as heat from PSII.
- **Energy-dependent quenching (qE):** The primary regulatory mechanism of energy dissipation in response to excess light, protecting the photosynthetic apparatus from photodamage.
- **Photoinhibition-related quenching (qI):** Refers to a sustained reduction in PSII efficiency that persists even after high-light exposure has ceased.
- **Quantum yield of non-photochemical quenching (Φ_{NPQ}):** Quantifies the effectiveness of protective mechanisms, such as the xanthophyll cycle, in dissipating excess light energy as heat.
- **Photochemical quenching (qP):** Estimates the fraction of PSII reaction centers that remain open and capable of accepting electrons.

2.6. Physiological Indices

- **Fresh and dry biomass:** Evaluated monthly, including dry biomass of roots, stems, leaves, and total biomass. Measurements were taken using an analytical balance after oven-drying samples at 75°C until a constant weight was achieved, indicating complete water loss.
- **Leaf area index (LAI):** The ratio of total leaf area to the ground area occupied by the plant.
- **Net assimilation rate (NAR):** A physiological parameter indicating the efficiency of the plant in converting assimilated energy into biomass, expressed as dry weight gained per unit of leaf area over a defined period (five months).
- **Relative growth rate (RGR):** The main metric in growth analysis, defined as the increase in biomass per unit of existing biomass and time.

- **Crop growth rate (CGR):** Also known as crop growth rate per unit soil area and time, calculated according to the equation proposed by Fageria et al. (2006).

Measurements were taken every eight days on five randomly selected plants within the experimental plot to monitor physiological plasticity changes in response to water stress treatments.

2.7. Biochemical Variables

To analyze the enzymatic activity of superoxide dismutase (SOD), phenylalanine ammonia-lyase (PAL), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX), lyophilized leaf samples from cocoa genotype CCN51 (*Theobroma cacao*) were collected at 0, 6, 12, 24, 48, and 96 hours. The plant tissue was ground in liquid nitrogen. Subsequently, 40 mg of macerated tissue were weighed on an analytical balance, followed by the addition of 0.07 g of PVPP per gram of tissue to prevent oxidation. The homogenate was then resuspended in 800 μL of extraction buffer (specific for each enzyme) and vortexed thoroughly.

Next, the samples were sonicated using a probe ultrasonic homogenizer (Gex 130, 130 W) at 70% amplitude, applying 10 pulses of 5 seconds each with 10-second intervals. Samples were then centrifuged at $14,000 \times g$ for 10 minutes at 4°C . The supernatant was transferred to a new microtube and considered the crude extract, which was used immediately for enzyme assays. Enzymatic activity was quantified using a SpectraMax Paradigm microplate spectrophotometer (Molecular Devices).

2.8. Statistical Analysis

The experiment followed a completely randomized design with a $1 \times 4 \times 15$ factorial scheme, where "1" corresponds to the genotype CCN51 used in this study, "4" to the four potassium treatments (100, 200, and 400 mg dm^{-3} of soil and a control with complete fertilization), and "15" to the number of replicates per treatment, consisting of 15 plants per experimental unit.

The data were subjected to analysis of variance (ANOVA), and treatment means were compared using Tukey's test ($p < 0.05$). In addition, a trend analysis was performed to assess the relationship between crop development and growth period (six months), allowing for the identification of phenological dynamics of plants under different potassium fertilization treatments.

3. Results and Discussion

3.1. Agronomic and Photosynthetic Variables

Figures 1, 2, and 3 display the results of the morpho-agronomic variables, specifically the number of leaves, plant height, and stem diameter, in the cocoa clone (*Theobroma cacao* L.) CCN51. These parameters were evaluated under water stress conditions and varying potassium (K) doses over a six-month period. The analysis was based on the visual interpretation of data trends represented in the graphs and supported by scientific literature, which aids in the interpretation of the physiological changes observed in the plants.



Figure. 1 Effect of Potassium Doses and Water stress on Plant Height in Cocoa Clone CCN51 Over a Six-Month Period.

The treatments showed significant differences in vertical growth: T0 reached a height of 25 cm, followed by T3, while T1 and T2 exhibited lower development (17–19 cm). These results indicate that high potassium (K) concentrations stimulated stem elongation, even under water stress conditions. The underlying mechanism involved the maintenance of osmotic potential and ionic homeostasis, allowing for the cell turgor necessary for vegetative expansion (Silva et al., 2023).

Treatment T3 exhibited a distinctive morphophysiological pattern, with sustained vertical growth despite a reduction in leaf area. This phenotype suggests an adaptive strategy of preferential

resource allocation to the main axis, possibly to optimize light interception under stress conditions. Parallel studies in *Coffea arabica* have shown similar effects, where applications of 145 kg ha⁻¹ of K significantly increased plant height and productivity (Siregar et al., 2023).

In contrast, potassium deficiency triggered restrictive physiological responses, including reduced stem elongation due to limited cell division and expansion (Zhao et al., 2016). Measurements using the MultispeQ device confirmed a dose–response relationship, showing that K availability modulated key photosynthetic parameters: plants with adequate potassium nutrition maintained higher values of ΦPSII (quantum efficiency of PSII) and qP (photochemical quenching coefficient), indicating better performance of the photosynthetic apparatus under stress.

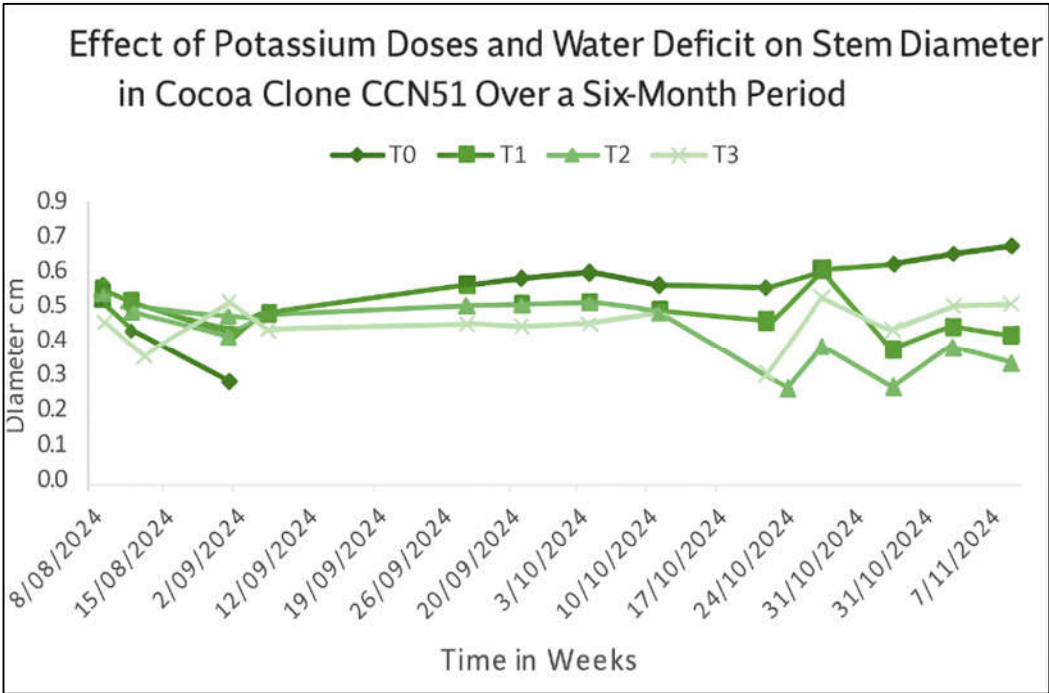


Figure. 2 Effect of Potassium Fertilization and Water stress on Stem Diameter in Cocoa Clone CCN51 Over a Six-Month Period.

Stem diameter showed notable variation among the evaluated treatments. Treatment T0 exhibited progressive and sustained thickening, reaching values above 0.85 cm at the end of the trial, reflecting favorable structural development. In contrast, treatments T2 and T3 consistently

recorded lower diameters, remaining below 0.6 cm on most of the evaluated dates, indicating a limited response to the applied nutritional management. Potassium, as an essential macronutrient, played a key role in the growth and development of *Theobroma cacao*. The superior performance observed in T0 suggested greater efficiency in the internal transport of water and nutrients, which favored biomass accumulation in supporting structures.

On the other hand, treatments T2 and T3 showed physiological constraints that negatively affected stem thickening, possibly due to potassium over-fertilization or negative interactions with other nutrients. These results are consistent with previous findings, such as those reported by Amoah et al. (2021), who found that potassium application increased stem diameter in various cocoa cultivars, even under water stress conditions. In this context, the increase observed in T0 may be associated with enhanced capacity for the transport of water and minerals, a critical condition for proper physiological development of the plant. Therefore, it is confirmed that a balanced potassium fertilization strategy is crucial for optimizing plant architecture and, consequently, for achieving the intended goals in terms of structural growth.

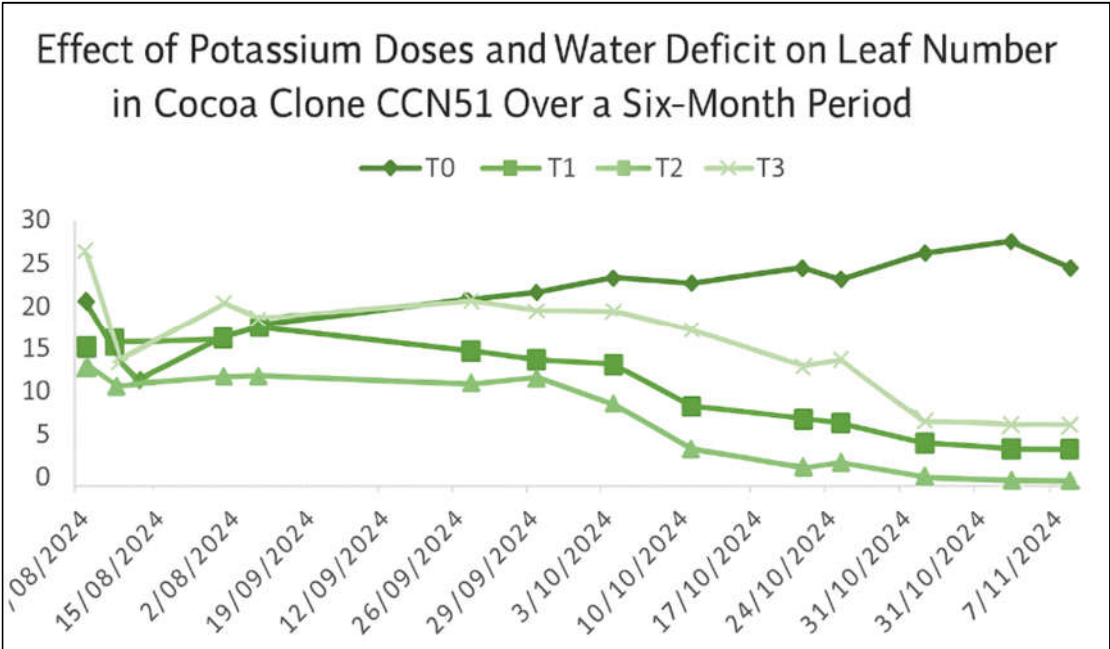


Figure. 3 Effect of Potassium Fertilization and Water stress on Leaf Number in Cocoa Clone CCN51 Over a Six-Month Period

Throughout the trial, treatment T0 (control, without potassium fertilization) exhibited a steady increase in leaf number, exceeding 28 leaves by the end of the evaluation period. This trend reflected a high rate of leaf emergence and canopy renewal, indicating active vegetative development even under water stress conditions. Such a response suggests that in the absence of potassium supplementation, the plants retained their leaf growth capacity, possibly by avoiding osmotic imbalances.

In contrast, treatments T2 and T3, which received the highest potassium concentrations, showed a sharp decline in leaf number beginning in the third week, stabilizing at low values between 3 and 7 leaves. This pronounced reduction in leaf production indicates a significant physiological adjustment, in which plants reduced their leaf area as an adaptive strategy to cope with the combined stress of potassium excess and water stress. This response aligns with findings by Taiz and Zeiger (2015), who noted that under adverse conditions, plants tend to minimize leaf surface area to limit transpiration and conserve energy.

Physiological measurements using the MultispeQ device (PhotosynQ platform) confirmed that the reduction in leaf production was not solely a morphological effect but also a metabolic adaptation aimed at reducing water and energy demand. This adjustment is consistent with observations by Rivas et al. (2020), who demonstrated that drought stress triggers systemic responses that impact photosynthetic activity and vegetative growth.

Similarly, in species such as *Theobroma grandiflorum* (cupuaçu) and *Euterpe oleracea* (açai), recent studies have shown that leaf emergence is strongly influenced by hormonal balance and nutrient availability—particularly potassium—which directly affects foliar cell division (González et al., 2019). Therefore, it can be inferred that treatments T2 and T3 induced foliar growth inhibition as a water-conservation mechanism, while T0 enabled a more efficient vegetative response under stress conditions, thereby supporting the objective of assessing the interaction between potassium fertilization and water stress in cocoa clones.

3.2 Photosynthetic Variables

The physiological analysis based on photosynthetic variables (Table 2) enabled a more precise assessment of the functional responses of cocoa plants to the different treatments by identifying

internal processes such as electron transport, energy dissipation, and the photochemical efficiency of the photosystems. In this study, key parameters were evaluated (Table 3), including proton conductivity of ATP synthase (gH^+), linear electron flow (LEF), steady-state fluorescence (F_s), and other indicators related to the quantum yield of both PSII and PSI.

Statistical analyses, including ANOVA and Tukey’s multiple comparison test, revealed significant differences among treatments, providing a detailed understanding of the physiological behavior of the plants under varying experimental conditions.

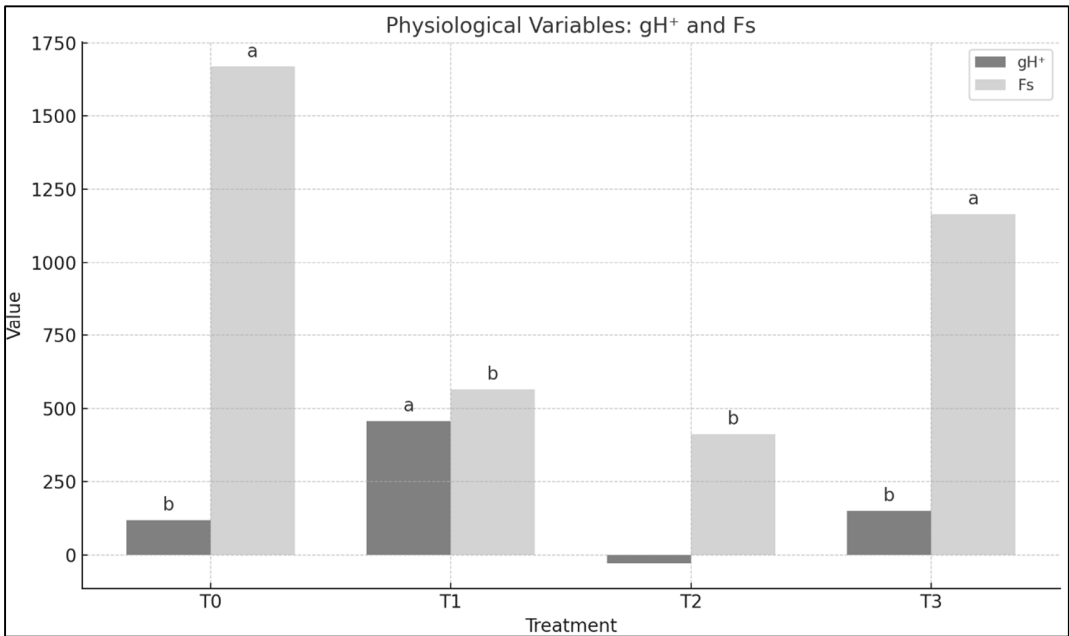


Figure. 4 Effect of Potassium Treatments on Proton Conductivity (gH^+) and Steady-State Fluorescence (F_s) in Cocoa Clone CCN51

The results obtained in this study using the MultispeQ v2.0 device revealed differentiated physiological responses in variables associated with electron transport efficiency in cocoa clones subjected to various treatments. Notably, treatment T1 showed the highest value of proton conductivity (gH^+), approximately 500, indicating greater efficiency in the establishment of the proton gradient and, consequently, more effective ATP synthesis via ATP synthase activity. This behavior aligns with findings reported by Kramer and Evans (2011) and validated in recent studies on coffee and cocoa crops (Magney et al., 2019; Cardona et al., 2021), where increased gH^+ was

associated with enhanced photosynthetic capacity under favorable light and water availability conditions.

In contrast, treatment T2 exhibited the lowest gH^+ values, close to zero, suggesting potential dysfunction in ATP synthase activity, possibly induced by water stress or nutrient limitations. This physiological pattern is consistent with the observations of Torres et al. (2020) under moderate salinity conditions, where a reduction in the proton gradient was accompanied by decreased electron transport efficiency and increased production of reactive oxygen species. Therefore, the data suggest that T1 enhanced chloroplast bioenergetics, while T2 resulted in a severe metabolic limitation.

Regarding chlorophyll fluorescence (Fs), statistically significant differences were observed among treatments ($p < 0.05$). Treatment T0 recorded the highest value (around 1700), followed by T3 (approximately 1200), indicating greater efficiency in light energy capture and lower reliance on dissipative mechanisms. These results are consistent with those reported in shaded coffee plantations, where higher Fs values were associated with optimal light conditions and minimal thermal stress (Magney et al., 2019). Consequently, clones under T0 and T3 may have maintained a more functional and efficient photosynthetic apparatus under the evaluated conditions.

Conversely, treatments T1 and T2 exhibited significantly lower Fs values, indicating a state of mild to moderate photoinhibition or the activation of non-photochemical quenching mechanisms as a protective strategy against environmental stress. This behavior is in line with reports from cocoa trials under salinity and water stress conditions, where reduced Fs and increased ΦNPQ were observed as part of the plant's photoprotective response (Avellaneda-Torres et al., 2020; Zivcak et al., 2016). Although no significant differences were detected in ΦI2 among treatments, there was a slight trend toward lower values in T2, consistent with its low Fs levels. This reduction suggests moderately compromised photochemical efficiency, though without a critical impact on PSII performance.

Overall, the data support the conclusion that treatments T0 and T1 elicited contrasting yet relevant physiological responses: T0 maintained high light-harvesting efficiency (Fs), while T1 promoted a stronger proton gradient (gH^+). In contrast, T2 was associated with impaired physiological function, reinforcing the importance of evaluating multiple photosynthetic indicators to understand

the biochemical and energy efficiency impacts of treatments on cocoa under climate change scenarios.

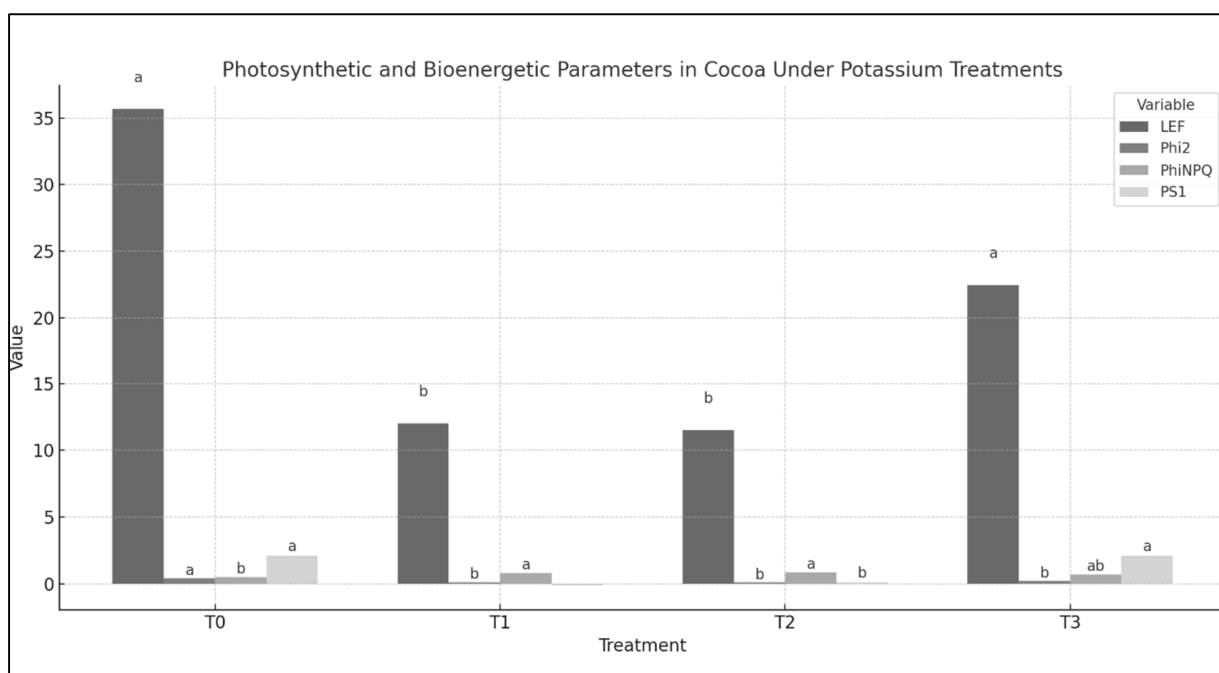


Figure. 5 Effect of Potassium Fertilization on Photosynthetic and Bioenergetic Parameters in Cocoa Clone CCN51 Under Water stress Conditions

The analysis of linear electron flow (LEF) revealed that, although no statistically significant differences were found between treatments ($p > 0.05$), treatment T2 exhibited the lowest values, indicating: reduced efficiency in electron transport, a negative impact on photosynthetic capacity, and a possible limitation due to water availability. These results are consistent with findings in *Theobroma grandiflorum*, where non-photochemical quenching mechanisms stabilized LEF under environmental variation (Rivera et al., 2019). The integration of parameters such as proton conductivity (gH^+) and linear electron flow (LEF) enabled a comprehensive physiological assessment, which is essential for early stress detection. Studies in *Euterpe oleracea* have validated

this methodological approach, demonstrating the utility of the MultispeQ device in optimizing agronomic practices (Silva-Santos et al., 2022).

Regarding Φ PSI (quantum efficiency of photosystem I), the analysis showed: no significant differences among treatments, slightly higher values in T1 and T2, and overall functional stability of photosystem I. This response aligns with reports on *Theobroma grandiflorum*, where compensatory mechanisms maintained stable Φ PSII under variable conditions (Rivera et al., 2019). The observed stability suggests: high photosynthetic plasticity in the evaluated clones, the ability to sustain photosynthetic function under stress, and adaptive traits characteristic of tropical perennial species (Yamori et al., 2016). These mechanisms helped offset variations in steady-state fluorescence (Fs), thereby maintaining productivity under suboptimal conditions.

In the case of Φ NPQ—a variable representing the proportion of absorbed light energy dissipated as heat—higher values were recorded in treatment T2, indicating a greater activation of thermal protection mechanisms under potentially adverse conditions. Although the differences were not statistically significant, the observed trend suggested an adaptive photoprotective adjustment. This behavior aligns with findings reported by Silva-Santos et al. (2022) in *Euterpe oleracea* (açai) and by Cardona et al. (2021) in coffee (*Coffea arabica*), where increases in Φ NPQ were associated with high irradiance or water stress. This response led to the redistribution of absorbed energy toward non-productive pathways, such as thermal dissipation, thereby protecting the photosynthetic apparatus from photooxidative damage. Zivcak et al. (2016) described this mechanism as a key component of the photochemical dynamics in plants exposed to environmental stress.

Lastly, the proportion of oxidized PSI centers showed no significant differences among treatments, indicating that the electron flow through photosystem I remained stable. This stability suggests that PSI retained its redox functionality even under stress conditions, contributing to the overall energy balance of the photosynthetic system. Similar results were reported by Magney et al. (2019) in cocoa and coffee, where PSI exhibited high functional tolerance under moderate stress. In the context of this study, such behavior represents a key physiological advantage, enabling the plants to sustain efficient electron transfer processes—an essential requirement for maintaining photosynthesis and crop productivity under scenarios of climatic variability.

3.3 Physiological Variables.

Figures 6 and 7 illustrate that plant biomass is a key indicator of physiological development, reflecting the plant’s ability to convert available resources (water, light, and nutrients) into structural growth. Both fresh biomass (FB) and dry biomass (DB) are essential for assessing the effects of factors such as water and nutrient availability—particularly potassium—on metabolic efficiency and stress tolerance. This section discusses the effects of the applied treatments (T0, T1, T2, and T3), corresponding to different combinations of potassium availability and water stress, on the accumulation of FB and DB in roots, stems, and leaves.

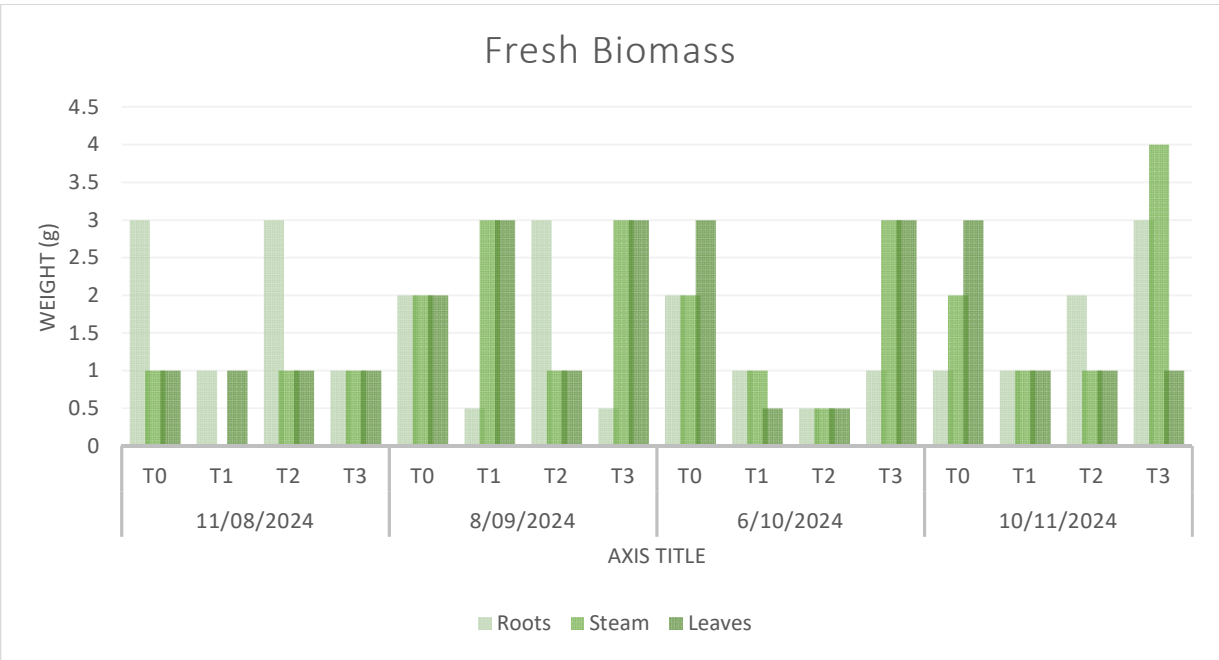


Figure. 6 Temporal Dynamics of Fresh Biomass Accumulation in Roots, Stems, and Leaves of Cocoa Clone CCN51 Under Potassium and Water stress Treatments

Dry biomass, by excluding water content, allowed for a more accurate assessment of the actual partitioning and accumulation of organic matter in plant structures. In this context, Figure 6 revealed a trend consistent with fresh biomass, with treatment T3 standing out as the one with the highest total dry biomass (DB) accumulation, particularly in leaves and roots, across all evaluation dates. This pattern carries important physiological implications, as the greater DB accumulation in T3 was associated with enhanced functional plant performance resulting from the positive interaction between potassium nutrition and water availability. This synergy supported key physiological processes such as stomatal opening, photoassimilate transport, and the biosynthesis

of structural compounds—factors that have also been described in tropical perennial species (Silva-Santos et al., 2022).

The use of the portable MultispeQ sensor, connected to the PhotosynQ platform, enabled the establishment of direct correlations between physiological variables—such as chlorophyll fluorescence (Fs), quantum yield of photosystem II (Phi2), and non-photochemical quenching (PhiNPQ)—and dry biomass accumulation. These findings are consistent with those reported by Kuhlert et al. (2016) in cocoa and coffee studies, supporting the hypothesis that photosynthetic efficiency is directly related to biomass accumulation under field conditions.

The results obtained for treatment T3 reaffirm the essential role of potassium (K) in regulating carbon metabolism. This element not only activates key enzymes involved in photosynthesis but also contributes to maintaining cellular water potential and promoting stomatal opening, thereby optimizing gas exchange and water use efficiency (Wang et al., 2016). Under the mild water stress conditions evaluated in this study, the presence of K sustained photosynthetic activity and the synthesis of organic compounds, which translated into greater dry biomass accumulation in T3.

Studies with *Euterpe oleracea* (açai) seedlings under different potassium doses reported similar responses, showing increased shoot and root biomass as K supply increased, associated with more efficient photosynthesis (Silva et al., 2022). This parallel supports the notion that the effect of potassium on biomass accumulation can be extrapolated to other economically important perennial crops.

Treatments T1 and T2 showed intermediate levels of dry biomass accumulation, suggesting that both irrigation and potassium fertilization had individually positive effects. However, these effects did not reach the levels achieved by T3, underscoring the importance of the interaction between both factors. In contrast, treatment T0 exhibited the lowest dry biomass accumulation across all time points, reflecting the physiological limitations imposed by combined water and nutrient deficiency.

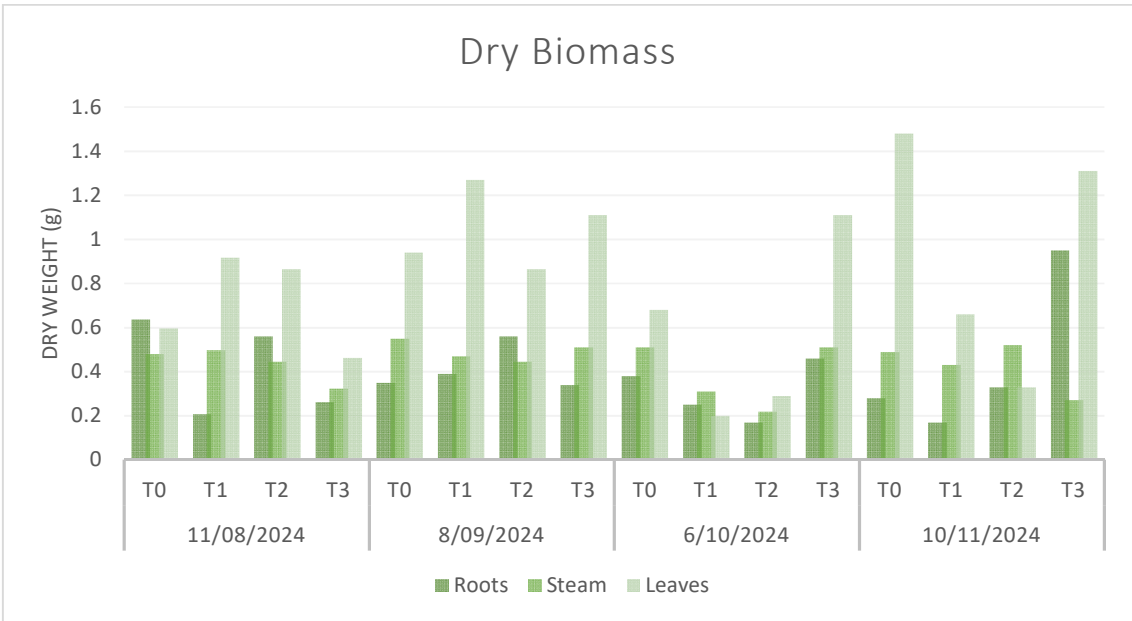


Figure. 7 Dry Biomass Partitioning in Roots, Stems, and Leaves of Cocoa Clone CCN51 Under Potassium and Water stress Treatments Over Tim

Data from Figure 7 revealed that treatment T3 (high potassium availability with adequate irrigation) consistently exhibited the highest fresh biomass values across all evaluated organs (roots, stems, and leaves), reaching its peak development on 11/10/2024. This response highlighted the synergistic effect between potassium nutrition and water management, which promoted optimal physiological balance, increased stomatal conductance efficiency, and enhanced photosynthetic activity. These findings are consistent with previous studies in *T. cacao* and *Coffea arabica*, where the potassium–water interaction significantly improved both photosynthetic efficiency and biomass accumulation (Cardona et al., 2021). The observed synergy enabled a more efficient and resilient metabolic function in the face of environmental variability.

Treatment T0 (no potassium and no irrigation) showed the lowest fresh biomass values across all plant organs, reflecting limitations in ATP synthesis and deficiencies in sugar transport. This response aligns with the findings of Wang et al. (2016), who reported that combined water and nutrient deficits drastically reduce photosynthetic efficiency by impairing PSII activity and cellular expansion processes. Measurements obtained using the MultispeQ confirmed this metabolic stress, with treatment T0 showing reduced Φ PSII values and elevated Φ NPQ (Torres et al., 2020).

Treatment T1 (potassium fertilization without irrigation) showed intermediate improvements compared to T0, but biomass levels remained lower than those in T2 and T3, indicating a limited physiological response. These results demonstrate that, although potassium modulates stomatal dynamics, its effectiveness critically depends on water availability to maintain cell turgor, sustain enzymatic activity, and facilitate solute transport (Rai et al., 2017).

This study demonstrates that only the optimal combination of potassium nutrition and water management (T3) ensures full physiological development, maximum biomass accumulation, and resilience to environmental stress—supporting the need for integrated fertilization and irrigation strategies to optimize cocoa productivity under variable conditions.

Figures 8, 9, 10, and 11 present physiological growth indicators including Net Assimilation Rate (NAR), Relative Growth Rate (RGR), Crop Growth Rate (CGR), and Leaf Area Index (LAI). These parameters were essential for understanding the functional efficiency of the plants under different environmental and agronomic management conditions. These indices allowed for an integrated evaluation of the effects of potassium availability and water stress on biomass production, photosynthetic activity, and resource partitioning. The following section discusses the results obtained based on the applied treatments.

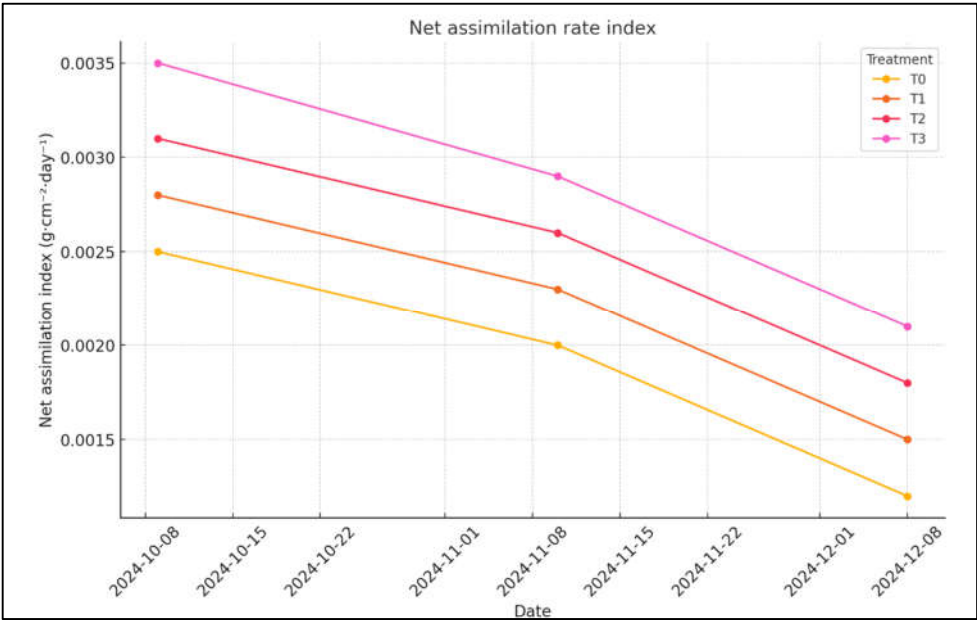


Figure. 8 Net assimilation rate index

The Net Assimilation Rate (NAR) reflects the efficiency with which plants convert light energy into biomass. As shown in Figure 8, treatment T0 (control) exhibited the highest peak on September 1, 2024, exceeding $0.04 \text{ g} \cdot \text{cm}^{-2} \cdot \text{day}^{-1}$. This indicated a momentarily high assimilation efficiency. However, this value declined sharply in subsequent evaluations, suggesting a transient response likely linked to an initial physiological reaction to water stress, followed by a rapid depletion of metabolic resources.

In contrast, treatment T3 displayed lower but more stable NAR values throughout the experimental period. This pattern suggested a more consistent and regulated photosynthetic response, attributable to the beneficial effect of potassium supplementation. Such physiological stability denotes a more resilient and efficient adaptation strategy under stress conditions, which is especially relevant for perennial crops like cacao. Several studies have supported the utility of the MultispeQ device in monitoring tropical crops such as cacao (*Theobroma cacao*), açai (*Euterpe oleracea*), and coffee (*Coffea arabica*), due to its ability to detect early physiological responses to environmental and nutritional fluctuations (Herrera et al., 2022).

In cacao, Díaz et al. (2019) reported a strong correlation between ΦPSII measured with MultispeQ and net assimilation rates under varying shade and water availability conditions. Similarly, Vélez et al. (2023) found that optimal potassium levels in coffee maintained stable ΦPSII values, reflecting improved biomass production efficiency closely associated with NAR.

In line with these findings, Aparecido et al. (2021) demonstrated in copoazú (*Theobroma grandiflorum*) that water stress significantly reduced NAR, an effect that could be mitigated through potassium fertilization and shading strategies. The results of the present study are consistent with this evidence: treatment T3 allowed for sustained NAR, implying a more efficient use of available resources and reduced physiological vulnerability to environmental variability. This trait is highly desirable in sustainable production systems, as it enhances crop growth stability and long-term productivity.

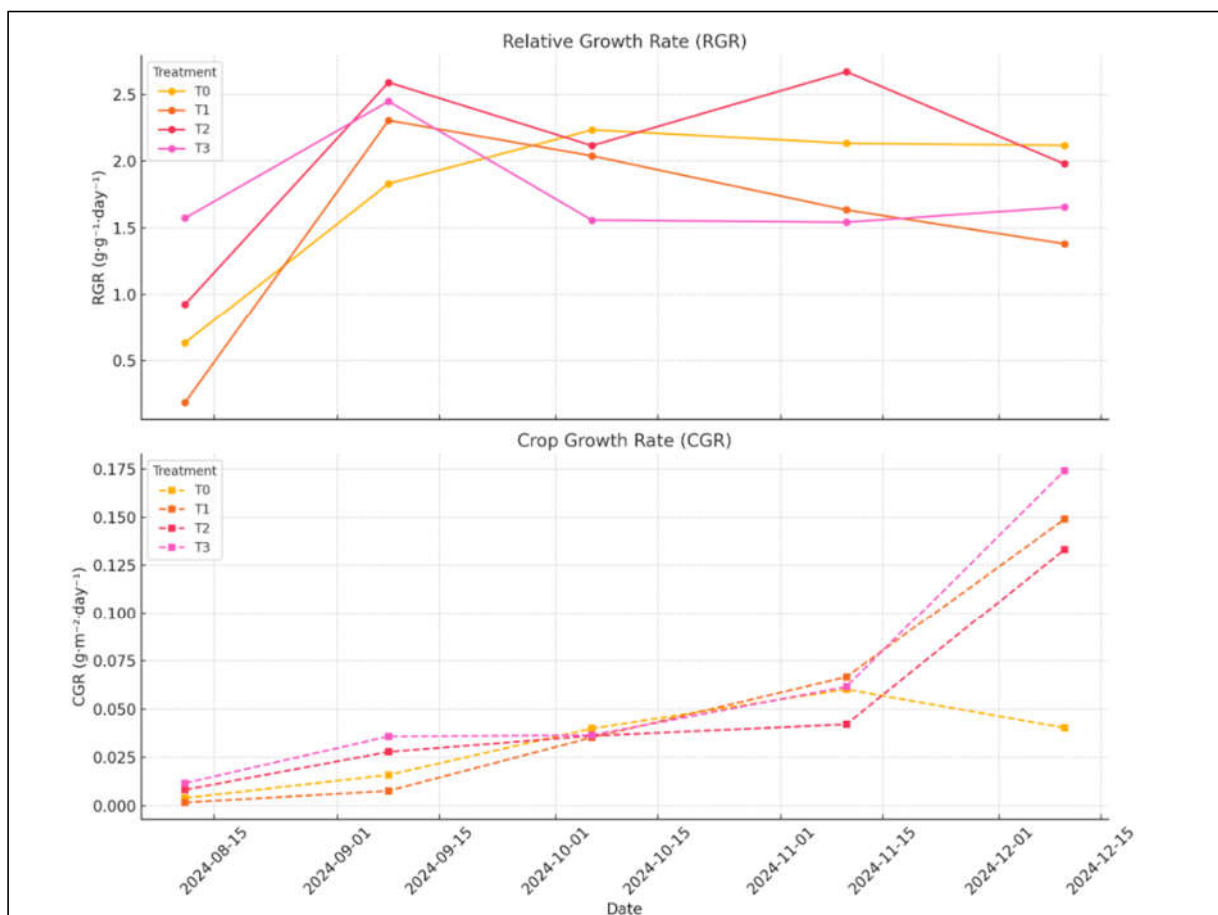


Figure. 9 and 10. Growth Dynamics of Cacao under Different Potassium and Irrigation Treatments.

Relative Growth Rate (RGR) reflected the plant's ability to increase biomass relative to its existing mass. As shown in Figure 9, treatment T1 peaked at ~5.3 on September 9, 2024, surpassing all others. However, this was transient, with a consistent decline in subsequent dates. In contrast, T2 and T3 exhibited more stable trends, with peaks in October and November, suggesting better adaptation to moderate water stress. This stability likely stemmed from improved metabolic regulation and maintenance of physiological functions.

T2 plants, which maintained a consistent RGR, also exhibited higher Φ_{PSII} and SPAD values during peak growth, indicating a direct relationship between photosynthetic efficiency and sustained biomass accumulation. Similar patterns have been reported in *Theobroma cacao* and *Coffea arabica*, where high quantum efficiency and balanced nutrition supported prolonged vegetative growth (Amoah et al., 2021). In *T. grandiflorum* and *Euterpe oleracea*, MultispeQ

proved effective for detecting early stress responses before visible symptoms (Rivas et al., 2020). In this study, the decline in ΦPSII in T1 aligned with the drop in RGR, validating the use of physiological markers as early indicators of growth limitations.

Crop Growth Rate (CGR), which integrates biomass accumulation and leaf area, revealed key differences in treatment performance. On December 1, 2024, T2 and T3 reached the highest CGR (~ 0.16 and ~ 0.18), while T0 recorded the lowest. This confirmed that the absence of irrigation and potassium drastically limited cumulative growth. The superior performance of T3, combining potassium and irrigation, promoted greater photosynthetic efficiency and biomass partitioning compared to the control (T1).

Higher CGR in T2 and T3 was associated with increased chlorophyll content and enhanced electron transport in PSII, reflecting stronger biochemical responses under improved nutrient and water availability. These findings are supported by studies in tropical dicots: Silva et al. (2018) linked high SPAD and ΦPSII (measured with MultispeQ) to increased yield in *Coffea arabica* under optimal fertilization, aligning with our results for T2 and T3. Likewise, Amoah et al. (2021) showed that potassium fertilization under drought maintained ΦPSII and LEF in *T. cacao*, sustaining growth.

Comparable responses were observed in T1, which included potassium alone. Moreover, Rivas et al. (2020) found that balanced nutrition improved photosynthetic efficiency and CGR in *E. oleracea* within agroforestry systems. Collectively, these findings confirm that combined nutrient and water management enhances physiological and biochemical growth mechanisms, validating the approach taken in this study to mitigate drought stress in cacao clones.

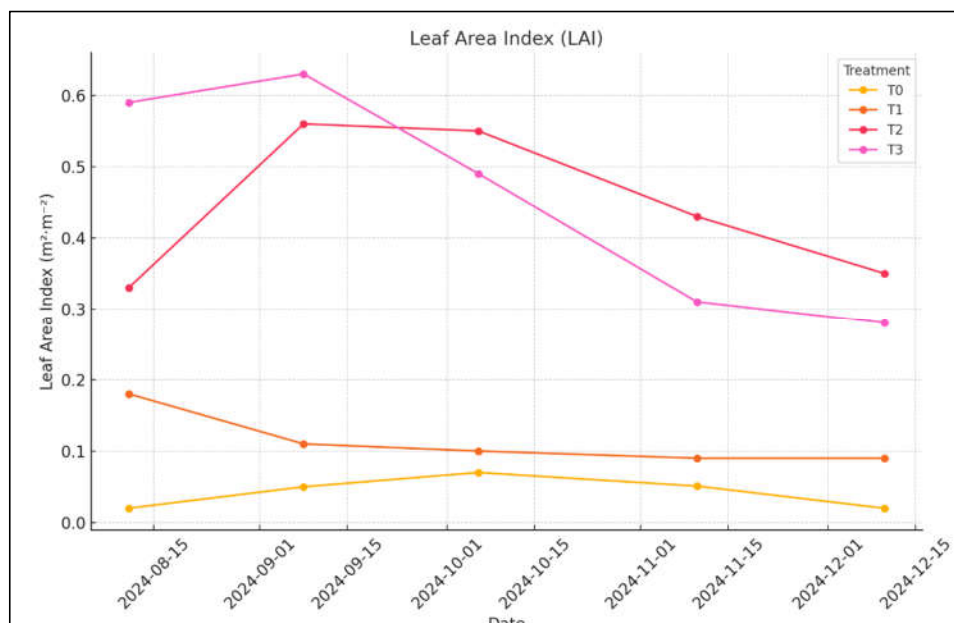


Figure. 10 Leaf Area Index – LAI

The **Leaf Area Index (LAI)** served as a key ecophysiological parameter in this study, representing the ratio of leaf surface area to total plant biomass and indicating the crop's potential photosynthetic capacity (Araújo et al., 2019). This index reflects the efficiency in capturing photosynthetically active radiation (PAR), a critical factor for productivity in perennial species such as cacao (*Theobroma cacao*), copoazú (*T. grandiflorum*), coffee (*Coffea arabica*), and açai (*Euterpe oleracea*) (Torres et al., 2021).

During the evaluation period, treatment T3 recorded the highest LAI, peaking at ~0.65 in September 2024, followed by T2 (~0.57). In contrast, T0 showed the lowest values throughout, never exceeding 0.07. The greater leaf development in T3 increased light interception and photosynthetic potential, enhancing biomass production. This aligns with findings by Almeida et al. (2020), who linked high LAI in copoazú with better solar energy conversion, provided no severe environmental constraints were present.

T2 and T3 also maintained relatively stable LAI values during mid-cycle (October–November), suggesting balanced resource allocation between leaf expansion and structural development—an adaptive pattern in crops under favorable soil and water conditions, as observed by Silva et al. (2017) and Villamil et al. (2019) in shaded cacao and açai agroforestry systems.

In contrast, T0's consistently low LAI reflected a conservative growth strategy under water or nutrient stress, prioritizing survival over expansion. This mirrors responses seen in drought-stressed coffee plants (Delgado et al., 2020). Overall, the enhanced physiological efficiency observed in T2 and T3 supports the study's objectives, highlighting the importance of potassium and water availability in improving cacao resilience and productivity under controlled conditions.

3.4 Biochemical Variables

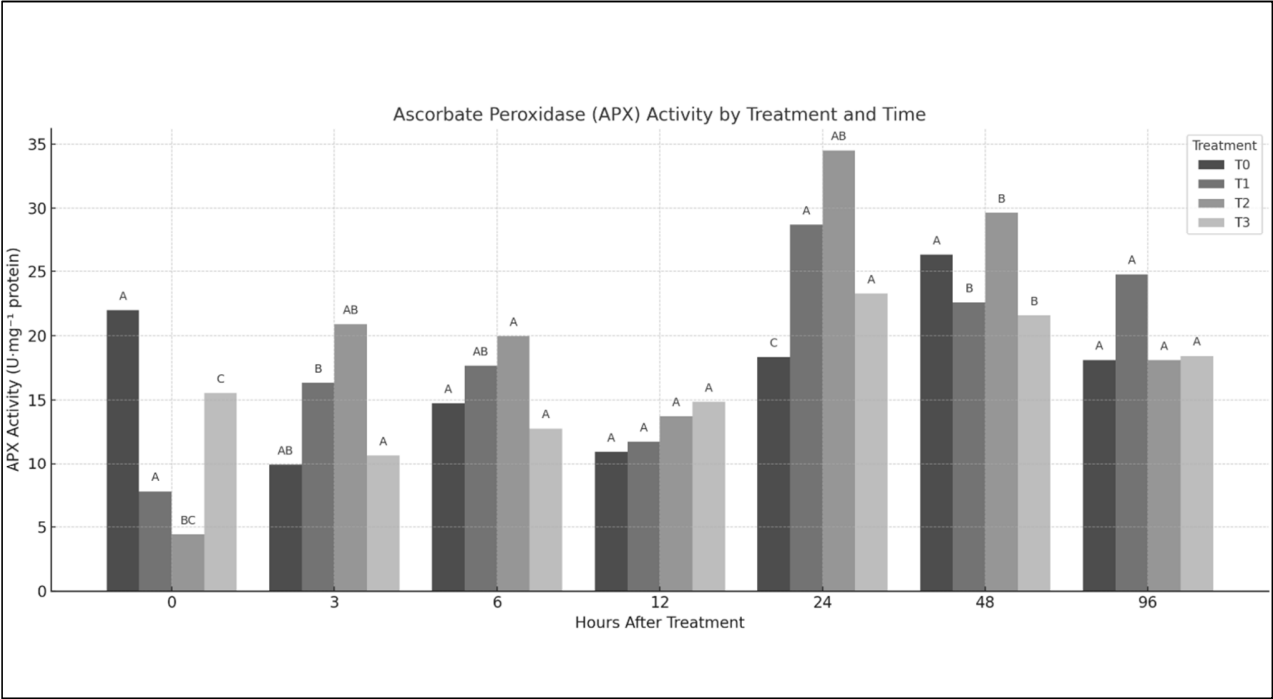


Figure. 11 Ascorbate Peroxidase (APX) Activity by Treatment and Time.

The ANOVA for ascorbate peroxidase (APX) activity revealed significant effects of treatment, time, and their interaction ($p < 0.05$), indicating a dynamic enzymatic response over time. At 24 hours, treatment T2 exhibited the highest APX activity ($>35 \text{ mmol ASA} \cdot \text{g}^{-1} \text{ FW} \cdot \text{min}^{-1}$), suggesting an enhanced antioxidant response under combined potassium supplementation and adequate water availability. This peak in APX coincided with higher ΦPSII values and reduced reactive oxygen species (ROS) accumulation, highlighting the critical role of potassium in modulating both antioxidant defense and photosynthetic efficiency in CCN51 cacao clones. The data support the hypothesis that balanced water and nutrient status strengthens the plant's oxidative stress response.

Furthermore, differences among treatments underscore potential genotypic variability in antioxidant capacity, consistent with previous findings in cacao (Torres et al., 2017) and coffee (Pérez et al., 2018). Overall, these results confirm the importance of potassium fertilization under moderate water stress and provide physiological evidence of its role in enhancing biochemical mechanisms linked to drought tolerance in cacao.

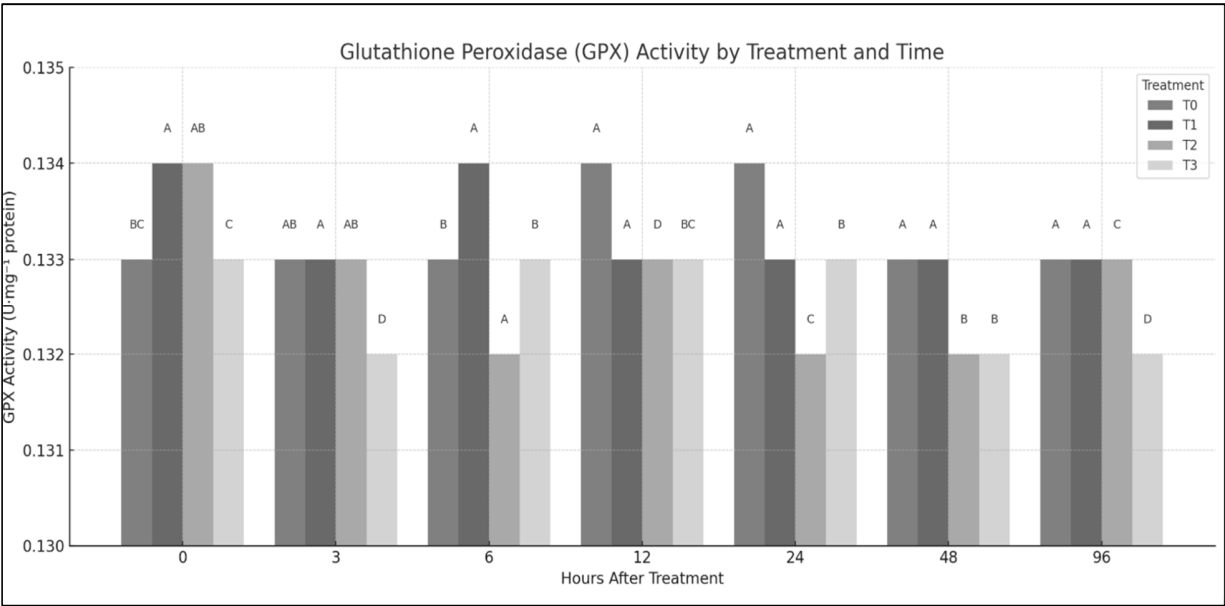


Figure. 12 Glutathione Peroxidase (GPX) Activity By Treatment And Time.

Glutathione Peroxidase (GPX) activity remained stable over time, showing limited responsiveness to potassium fertilization or water stress. ANOVA revealed that significant differences were mainly associated with the factor "time," while no major differences were

observed among treatments ($p > 0.05$). Enzymatic activity values ranged narrowly between 0.12 and 0.14 $\mu\text{mol DTNB/min/g}$, confirming a steady response throughout the experiment.

This trend suggests that GPX plays a more constitutive role in the antioxidant defense system, functioning as a basal protective enzyme rather than reacting dynamically to environmental stressors. Similar findings were reported by Müller et al. (2023), who noted that GPX contributes to redox homeostasis without exhibiting sharp fluctuations under stress.

In contrast to APX, whose activity varied significantly with potassium availability and drought conditions, GPX remained largely unaffected—indicating its regulation is likely governed by internal cellular signals rather than external inputs. This was further supported by physiological measurements using the MultispeQ device, which detected early signs of stress (e.g., declines in ΦPSII and stomatal conductance) before any notable change in GPX activity was evident. These observations align with studies in cacao clones under drought (Jiménez et al., 2019), where GPX activity stayed constant while other physiological parameters declined. Overall, our results confirm GPX’s role as a stabilizing component of the antioxidant system, minimally influenced by fertilization, and crucial for maintaining cellular equilibrium during prolonged stress.

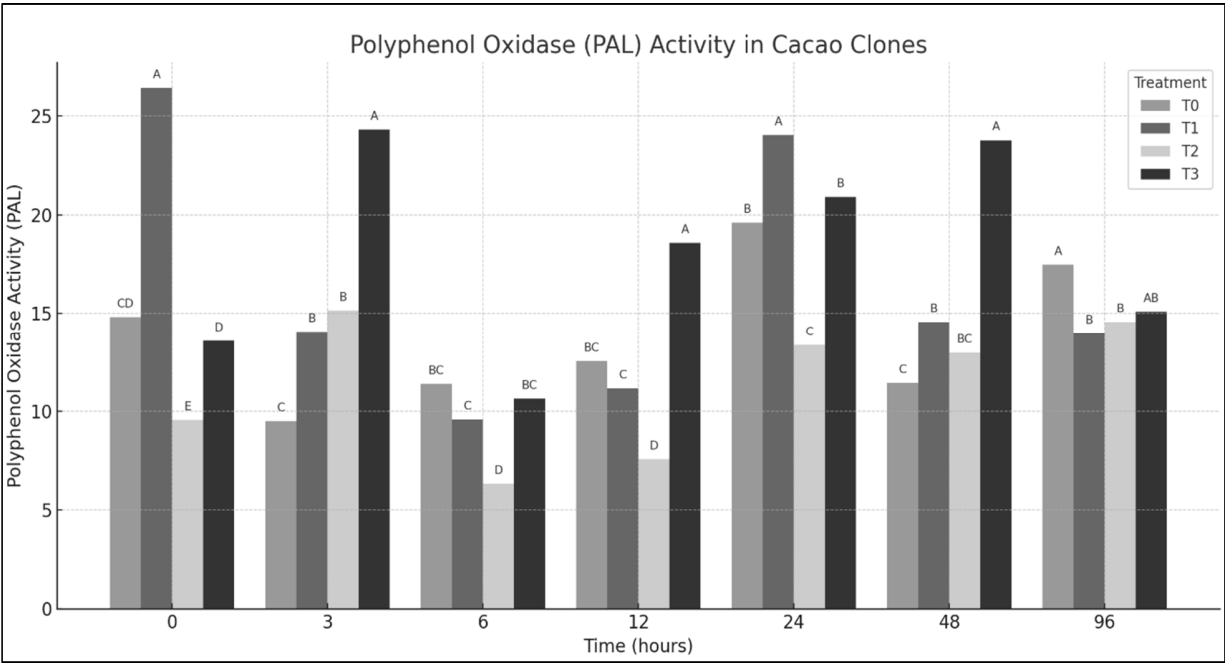


Figure. 13 Polyphenol Oxidase (PAL) Activity In Cacao Clones.

The enzymatic activity of phenylalanine ammonia-lyase (PAL) played a key role in the defense response of the evaluated cacao clones, by catalyzing the conversion of phenolic compounds into quinones—molecules with antimicrobial properties that also facilitate lignin cross-linking, reinforcing cell walls against pathogen invasion. In this study, PAL activity showed statistically significant differences due to both treatment and evaluation time, with a notable interaction between the two factors ($p < 0.05$).

Treatments T1 and T3 exhibited the highest PAL activity at 0 and 24 hours ($>25 \text{ mmol} \cdot \text{h}^{-1} \cdot \text{g}^{-1} \text{ DW}$), suggesting a rapid and effective activation of the defense system. This early response likely reflects a heightened secondary metabolism under stress conditions, consistent with findings in tropical crops such as cacao, where PAL is triggered by pathogens like *Phytophthora* and *Moniliophthora*, particularly when potassium (K) is adequately supplied. Potassium is known to support the biosynthesis of secondary metabolites under both biotic and abiotic stress (Hidalgo et al., 2020).

The use of the MultispeQ device, integrated with the PhotosynQ platform, enabled non-destructive monitoring of physiological traits including PSII quantum efficiency (ΦPSII), stomatal conductance, electron transport rate (LEF), and relative chlorophyll content. When combined with PAL activity measurements, this approach provided a comprehensive view of the physiological and biochemical plant status under stress. In cacao, decreases in ΦPSII under fungal infection have been correlated with increases in PAL activity and quinone accumulation, indicating a link between photosynthetic performance and oxidative defense activation (Aguiar et al., 2020). Similarly, in *Euterpe oleracea*, saline stress was shown to increase PAL activity in parallel with LEF and chlorophyll content (Souza et al., 2021).

In *Theobroma grandiflorum* (cupoazú), PAL has been recognized as an early biomarker of water stress, particularly when associated with reductions in ΦPSII and increases in leaf reflectance, aiding timely agronomic decisions (Córdova et al., 2022). Overall, the elevated PAL levels in potassium-treated plants suggest that K not only supports osmotic regulation but also modulates biochemical defense pathways, enhancing plant resilience. These findings align with prior evidence (Torres et al., 2017) and reinforce potassium fertilization as a strategic tool to induce systemic resistance in cacao under stress conditions.

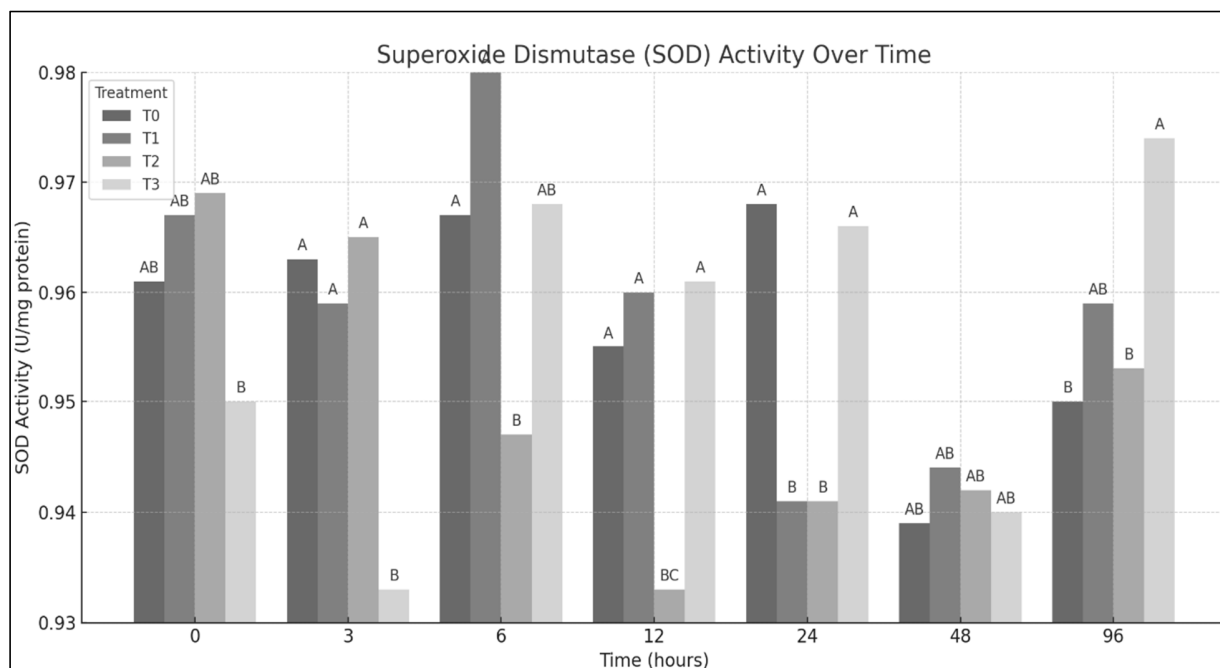


Figure. 14 Superoxide Dismutase (SOD) Activity Over Time

SOD represents the first line of defense within the enzymatic antioxidant system, catalyzing the dismutation of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), which is subsequently detoxified by catalase and peroxidase enzymes (Noctor et al., 2018). In this study, ANOVA revealed no statistically significant differences in SOD activity among treatments or across time points. This stability was reflected in the graphical analysis, with all treatments maintaining values close to $1.0 \text{ U} \cdot \text{mg}^{-1} \text{ DW}$ throughout the experimental period, indicating a tightly regulated enzymatic response.

These findings suggest that SOD activity is subject to strong post-translational or feedback regulatory mechanisms that buffer its fluctuations, even under induced drought stress. This aligns with previous studies reporting the conserved behavior of SOD under environmental perturbations (Hasanuzzaman et al., 2020) and its limited responsiveness when approaching saturation thresholds (Hernández et al., 2019). Moreover, in *Coffea arabica*, SOD activity was shown to remain stable despite significant changes in photosynthetic parameters under water stress (Menezes et al., 2021). Similarly, in *Theobroma cacao*, early alterations in $\Phi PSII$ and linear electron flow (LEF) preceded shifts in antioxidant gene expression, highlighting the photosynthetic response as an earlier indicator of redox imbalance (Aguiar et al., 2020).

Conclusions

Potassium × Water Synergy Drives Growth under Drought

The combined application of 30 g KCl plant⁻¹ (≈ 400 mg K kg⁻¹ substrate) plus supplemental irrigation (T3) produced the greatest physiological stability in CCN51 cacao. Compared with the unfertilised, non-irrigated control, T3 increased the crop growth rate by +260 % (0.18 g m⁻² day⁻¹, $p < 0.05$) and the leaf-area index by +828 % (0.65 vs 0.07), confirming potassium as a key determinant of canopy development and carbon gain even under moderate water stress.

Enhanced Bioenergetics under Optimal K Nutrition

Plants receiving adequate K maintained a proton-conductivity (gH⁺) of ~ 500 and a quantum efficiency of PSII ($\Phi_{PSII} > 0.70$), while K-deficient, water-stressed plants showed gH⁺ ≈ 0 and a marked drop in Φ_{PSII} . These results indicate that potassium preserves the thylakoid proton gradient, sustains ATP synthase activity and safeguards linear electron flow—mechanisms essential for continued photosynthesis during drought.

Antioxidant Defence Is Rapid and Dose-Dependent

Potassium supplementation sharply boosted ascorbate peroxidase (APX) activity to 35 mmol ASA g⁻¹ FW min⁻¹ at 24 h, curbing reactive oxygen accumulation. In contrast, superoxide dismutase (SOD) and glutathione peroxidase (GPX) remained constitutively stable, underscoring APX as a dynamic, K-inducible shield, while SOD/GPX provide basal redox homeostasis.

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Contributions

First author: conceptualization, methodology, investigation, data analysis, original draft writing, and project logistics. *Second author:* funding acquisition, project administration, supervision, methodology, and investigation. *Third to fifth author:* field logistics and data collection.

Data accessibility statement

IJANR is an open-access journal which promotes open and reproducible scientific research. If dataset will be available, detail which datasets associated with this submission are or will be archived, including accession numbers and/or DOIs, as available. IJANR has a community in Zenodo, so it is possible to upload datasets and get a DOI associated to the journal (<https://zenodo.org/uploads/new?community=ijanr>) before submission.

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