Ecophysiology and Nutrition of Cocoa

Lucette ADET

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Propositions

1. Fertilizer application is more effective to increase cocoa yield when other limiting factors are alleviated.
   (this thesis)

2. Developing drought-tolerant plants is a tough way to fight drought.
   (this thesis)

3. The decrease in rainfall may lead to more global conflicts over water.

4. Open communication with supervisors significantly impacts a student's academic success.

5. Knowledge that is not regularly updated or revisited may become less relevant.

6. Motivational dreams are a prerequisite to deal with stress during the PhD journey.

Propositions belonging to the thesis, entitled

Ecophysiology and nutrition of cocoa

Lucette Adet
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Ecophysiology and Nutrition of Cocoa

Lucette Adet

Thesis

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To my lovely daddy,

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Abstract

Cocoa production faces significant challenges due to climate change, particularly higher variability in rainfall patterns, increasing drought events, and increasing air temperature. In the West African cocoa belt, where farms manage aging cocoa trees with stagnant production, soils are becoming potassium (K) deficient due to the large K removal during harvesting. While K is believed to play an important role in the ability of crops such cocoa to tolerate drought stress, its effect on physiological processes in adult field-grown cocoa trees remains poorly understood.

This thesis provided an exploration of factors influencing cocoa tree performance, reproduction, and yield under water deficit field conditions in Côte d'Ivoire. It investigated the interaction between soil water deficit, K application, and genetic variations, offering insights into strategies to enhance cocoa resilience and yield in the face of climate change. The present research includes three studies in two contrasting experimental sites in different agro-ecological zones featuring two contrasting ways of manipulating water availability. The first experiment, presented in chapter 2, conducted in a relatively wet area in the Central part of Côte d'Ivoire's cocoa belt, studied drought effects through rainfall reduction on several physiological and reproductive traits as well as yield. This was done using rain shelters. Results demonstrated the influence of reduced rainfall on various aspects of cocoa growth and reproduction. The amount of rainfall water available to the plants was greatly reduced due to greater retention by the vegetation and losses to the atmosphere. Roots, physiological functioning, reproduction and yields responses were considerably reduced in drought condition. Drought conditions led to a substantial decrease in soil moisture by 9.1%, root length by ~57%, and root mass density by ~50%. It also adversely affected stomatal conductance by approximately 60%, leaf flush intensity by about 70%, and reduced leaf greenness and size by 48% and 68%, respectively. Consequently, these stressors led to diminished flower intensity and a decrease in healthy cherelles, pods, and bean numbers per tree, culminating in a yield reduction

from an average of ~2100 kg/ha to ~1450 kg/ha. K application increased Gs, leaf size and greenness particularly when soil moisture levels were adequate.

The second study, presented in chapter 3, assessed the effects of dry season irrigation and K application on adult cocoa trees, demonstrating significant impacts on various leaf physiology, morphology and crown traits. By including six genotypes we also explored the genotypic variation in these traits. Withholding irrigation decreased predawn and midday cocoa leaf water potential (LWP), stomata conductance (Gs) and sap flux rate. The LWP was 3 fold reduced in water stressed trees, implying that the water flux from soil to leaf was limited. Water deficit was responsible for ~ 23 % reduction in daily transpiration rate dropping from 2.92 to 2.37 L/Day. The hybrid genotype (M) exhibited greater increase in LWP, greater Gs and transpiration rate in response to irrigation than the clone (CI03). Moreover, the study found that leaf area, specific leaf area (SLA) and leaf water content (LWC) responses were significantly affected by irrigation. Additionally, applying K alongside watering had a beneficial impact on the moisture content of the leaves (LWC), enhancing their hydration levels. Leaf morphology responses to irrigation and K treatments were highly genotypes dependent. Crown density was reduced as the leaf area index (LAI) significantly decreased along with a lower light interception. Drought resulted in a 42% lower LAI, 15% lower light capture and a 17% greater water stress index (WSI). The effect of irrigation on these traits depended significantly on genotype which highlights the importance of understanding genetic variation for selecting drought-tolerant cocoa varieties. That would contribute to knowledge into cocoa acclimation strategies and resilient genotype selection.

The third study, in chapter 4, explored the interactive effects of seasonal irrigation, K application, and their impact on cocoa reproduction traits and yield. Results emphasized the role of these factors in improving cocoa yield, with K application enhancing pod development and contributing to increased annual yield. Irrigation, when combined with K application, almost doubled yield from 2000 kg/ha/year to 4000 kg/ha/year. This increase in yield was largely attributed to the beneficial impact of irrigation on the number of pods produced per tree. The effect of irrigation on yield depended on genotype, though our level of replication was insufficient to pinpoint variety was the most drought tolerant. Nonetheless, this irrigation by genotype interactive effect on yields points to a genotypic variation in drought tolerance that should be explored further. Together this thesis offers valuable information for enhancing cocoa productivity in the context of climate variability. It does so by providing a better understanding of how physiological systems are regulated to improve stress tolerance while maintaining sustainable cocoa yield. The results of this thesis emphasize the importance of considering combined strategies involving genetic diversity, irrigation practices, and nutrient management to ensure sustainable cocoa growth and yield under changing environmental conditions. Additionally, long-term studies could evaluate the sustainability of proposed irrigation and fertilization strategies, considering economic and environmental impacts. By translating these findings into actionable strategies, cocoa farmers can enhance the resilience of their crops.

Chapter 1

General Introduction

1.1 Côte d'Ivoire's cocoa urgency for a paradigm change: deforestation and sustainable initiatives

Côte d'Ivoire as the world's largest cocoa producer contributes over 40% of global cocoa bean production. The Ivoirian cocoa sector is valued at more than $4.7 billion per year which is more than one-third of the country's entire export revenue (OEC, 2020). However, the country faces severe deforestation linked to the cocoa industry, with an alarming 80% loss of forest cover over the last 60 years (Gockowski & Sonwa, 2011; FAO, 2017). The decrease in designated cocoa cultivation areas (also known as "cocoa-belt"), and the land degradation, affecting cocoa yields and soil fertility, highlight the need for sustainable practices (Tondoh et al., 2015).

The 2017 Cocoa and Forest Initiative (CFI), a collaboration between 35 companies and the Ivorian government, targets deforestation in the cocoa supply chain. Despite the 2017 CFI efforts', deforestation for the sake of cocoa cultivation is still ongoing. Cocoa cultivation areas have expanded drastically since the 1970s, intensifying deforestation and encroachment into protected areas (Goldman et al. 2020a; FAO 2020). An observed shift of the cocoa production from the south-east, where it was traditionally grown, to the south-west, has exacerbated deforestation and forest degradation in this new area (Chatelain et al., 2004; Wessel & Quist-Wessel, 2015). This shift occurred due to a rapid degradation of soil fertility and increasing incidence of pests and diseases.

Committed to a "zero deforestation objective," Côte d'Ivoire aims to restore forest cover and reconstitute millions of hectares of land by 2030 (BNETD, 2016). Yet, the shift from cocoa grown shade trees to full-sun and mono-cropping cocoa plantations initially beneficial for productivity, has led to significant ecological damage and unsustainable practices (Ruf, 2001; Koko et al., 2013). The annual cocoa deforestation rates were estimated at 13,000 ha per year over 2001–2015, with recent findings indicating even higher rates (Pendrill et al. 2022a; Pendrill et al., 2022b). In theory a means to reduce deforestation and yet meet global demands for cocoa would be to increase per unit land area yields. Efforts to boost yields through fertilizers and other means are on the rise to counter the increasing demand for land. But such intensification however also potentially comes with a larger impact on the environmental e.g. pollution through run off of agro-chemicals and greenhouse gas emissions associated with their production. Meeting these production and ecological challenges requires further knowledge of different types of inputs drive production (Smoot et al., 2013;Renier et al., 2023). Together, these facts advocate for a change in paradigm.

1.2 Drought impacts and the need for environmentally controlled experiments

Climate projections indicate a rise in the average global temperature by 2°C to 5°C by 2100, this increase being at least twice as fast as the past century (IPCC, 2021). This warming trend accelerates evaporation, diminishing area of water bodies, desiccating soils and vegetation, hence exacerbating drought conditions. In addition, rainfall patterns are projected to become more erratic, leading to more intense and longer dry spells (Coumou & Rahmstorf, 2012; Osborn et al., 2016). The agricultural sector bears the brunt of this climate variability, facing stress and crop damage, leading to diminishing yields and economic challenges as it is the largest anthropogenic consumer of water (Seneviratne et al., 2010; Naumann et al., 2018; Mcdowell et al., 2020; Seleiman et al., 2021).Drought-induced crop losses, in turn, contribute to food scarcity and higher food prices, exacerbating economic concerns (Tscharntke et al., 2011; W. M. O. et al., 2013; Sonwa et al., 2014; Niether et al., 2020).

The deliberate alterations of environmental factors such as rainfall patterns, would be an effective way to understand the effects of these changes on agro-ecosystems. However, such experiments are rare, especially in Africa, as they are often costly. These manipulations can take various forms including adjustments to variables such as temperature, carbon dioxide levels (as in Free-Air Carbon Dioxide Enrichment or FACE experiments), and water availability through irrigation or throughfall-exclusion experiments. Given the increased sensitivity of the region to climate variability, an increased understanding through area-specific and species-specific studies is essential for formulating effective adaptation strategies (Wangdi et al., 2017). Recent advancements in plant physiological studies on drought responses emphasize the need for further research in this domain (Korner, 2003; McDowell et al., 2008; Moser et al., 2010; Rowland et al., 2015; Niether et al., 2018).

1.3 Climate impacts on West African cocoa production

West Africa is projected to experience increased water deficit due to climate variability, which will likely negatively affect the production of agricultural commodities (Läderach et al., 2013) among which cocoa (*Theobroma Cacao L.*). The issue is aggravated by the rapid population growth in this part of the world (Herrmann & Brandt, 2013). As already discussed in the section "*Côte d'Ivoire's cocoa urgency….*", cocoa is of significant economic importance both for the producing countries such as Côte d'Ivoire and Ghana, and the consuming countries. Cocoa cultivation generates export revenues, income and employment. Nonetheless, while cocoa production plays a important role in the livelihoods of millions of farmers, the sector faces an increasingly uncertain future due to the potential impacts of climate variability (Medina & Laliberte 2017). Läderach et al (2011) and Läderach et al. (2013) initially forecasted a substantial decline in suitable areas. Currently, water availability is already the most limiting factor for cocoa growth and production (Anim-Kwapong & Frimpong 2004; Schroth et al., 2016). In recent years, variability in rainfall patterns have negatively affected cocoa yields, raising concerns about sustainable cocoa production under future climates. However, they later recognized that this prediction might have been too negative, as it did not account for adaptive agricultural practices and genetic factors (Läderach et al., 2013; Schroth et al., 2017).

Cocoa is grown mainly in developing countries, which could experience significant benefits from implementing more sustainable production practices. Among these benefits are increased yield, improved household income, enhanced working and living conditions, and better environmental protection (Ingram et al., 2018). Also, several agronomic strategies can be employed to make cocoa production less vulnerable to drought stress, including improved soil management through increased soil organic matter, nutrients availability, as well agroforestry, mulching and irrigation (Hutcheon et al., 1973; Abdulai et al., 2018b). Irrigation is the most obvious strategy to deal with drought conditions, but the implementation of irrigation technology requires relatively large investments, even when considering low-cost options (Otoo et al., 2018). It is not always feasible because of the economic costs and/or because of water sources inaccessiblility (Merrey & Lefore, 2018). The development and use of drought tolerant varieties could be another suitable strategy. The effectiveness of these strategies depend on understanding the physiological mechanisms behind drought tolerance in cocoa and the genetic variability therein, as well as the implementation of supportive policies. In this regard, it is critical to quantify and understand the genotypic variation in cocoa responses to low water availability. Unfortunately, to date relatively little research has been done on the effects of water availability on cocoa physiology, growth, phenology and yield. This knowledge is especially lacking for adult field-grown trees. This thesis aims to help bridge this knowledge gap by exploring the effects of variation in soil water availability on physiology and performance of field-grown cocoa trees and explore the genetic variation therein.

1.4 Cocoa ecophysiology

Cocoa is an evergreen, perennial tropical tree, grown for its edible seeds (Kongor et al., 2016) and cultivated in either full-sun monoculture system (Niether et al., 2020), within agroforestry system (Abdulai, et al., 2018b) or in thinned forests (de Almeida & Valle, 2007). Originally being a wet tropical forest species from the Amazon region, it is inherently adapted to thrive in humid tropical environments (Cheesman, 1944).

Ecophysiology is the field of study that deals with the physiological processes that regulate the interaction between organisms (in this case trees) and their environment, including water and nutrient uptake and use, gas exchange, and physiological responses to both biotic and abiotic stressors. In addition, ecophysiology also aims to quantify the effects of these stressors on plant performance and explores adaptation of plants to changing environments at the molecular, biochemical, physiological, and whole-plant levels (Marschner, 1995; Lambers et al., 2008).

Understanding the ecophysiology of cocoa plants, including their responses to environmental factors (water and nutrients deficit) is crucial for enhancing cocoa yield (Bridgemohan & Mohammed, 2019). This knowledge is particularly important given the limited use of irrigation and associated scope to directly reduce water limitations in cocoa farming across most of the major cocoa-producing countries. For instance, in West Africa such as Côte d’Ivoire, less than 1% of the land under cultivation is equipped with irrigation systems. Cocoa production in these countries is mostly rainfed (Asfaw & Lipper, 2016). Therefore, this thesis focusses on the ecophysiology of water limitation in cocoa and its subsequent effects on yield.

1.5 Role of soil water in physiological processes

Water plays a role in plant physiology due to its ability to: transport nutrients, maintain cell turgidity, and participate in various biochemical reactions (Edsall & McKenzie, 1978; Kramer & Boyer 1995). Water deficit constrains plant growth and crop yield to a greater extent than any other environmental factor (Boyer, 1982; Kramer, 1983). Crops - like all plants - use 95% of the water that they take up from the soil in the process of transpiration, which is a result of stomatal opening to let in CO_2 and has a cooling effect on leaves (Kramer, 1983). Plants predominantly absorb water and nutrients by their roots and this uptake mechanism is governed by both supply and demand factors at the root surface (Bederedse et al., 2007). Root traits may influence the movement of water from the soil to the leaves (Kramer, 1969) and are therefore obviously critical in crop growth and yield formation (Passioura & Angus, 2010). When root water uptake is no longer sufficient to meet the demand for transpiration, crops become water-stressed and their growth and yield are reduced. Furthermore, water and nutrient availability are also, to some extent, connected as nutrient movement into the soil and associated root nutrient uptake becomes more restricted in dry soil (Dubey & Pessarakli, 2001; Farooq et al., 2009). Finally, soil drying during a dry period occurs from the top towards the deeper soil layers with soil staying moist longer the deeper one goes. Deep rooting systems are therefore assumed to be an essential trait to support drought tolerance (Pinheiro et al., 2005; de Almeida & Valle, 2007).

1.6 Regulation of water loss: stomatal and leaf area control

Plants mitigate water loss by controlling stomatal opening, density and reducing leaf area. These mechanisms balance water loss with decreased soil water uptake (Sperry et al., 2002). Under low soil moisture, stomata react to drops in plant water potential, regulating internal water flow to stabilize water potential amid changing soil moisture and evaporation rates (Buckley, 2019). But the recovery time, the time needed for the plant to return to normal functioning once water stress has ended, differs between these response types. Plants can and adjust stomatal opening in two ways: by opening or closing their stomata or by producing more/larger or fewer/smaller stomata. Opening and closing of stomata typically occurs on a time scale of tens of minutes and is thus fastest way in which plants can adjust water losses to changes in water availability On the other hand, changes in number and size of stomata only occur when plants produce new leaves and a full adjustment at whole-plant level happens only after all leaves have been replaced (Buckley, 2005; Chaerle & Straeten, 2007). Thus, reductions in stomatal size and number and leaf area may take weeks to months to recover.

It is important to note that not all plant species follow the same strategy of stomatal control to drought stress. Plants are categorized according to the type of stomatal control; they exhibit either isohydry or anisohydry (Jones & Tardieu, 1998). Isohydric plants maintain relatively constant water potential when water availability is reduced; they tend to close their stomata relatively soon to limit water loss by reducing stomatal conductance and transpiration. On the other hand, anisohydric plants decrease water potential by keeping their stomata open longer (Sade et al., 2012; Hugalde & Vula, 2014). In addition to, stomatal density, size and aperture, drought tolerance has been associated with key leaf functional traits such as leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), and turgor loss point (TLP) in condition of stress (Farquhar et al., 2002; Poorter et al., 2009; Wright et al., 2021).

In general, cocoa trees tend to exhibit isohydric behavior, but their response to water deficit varies according to the genotype, environmental conditions, and the growth stage (Balasimha et al., 1991, Balasimha et al., 2013; Osakabe et al., 2014; Medina & Laliberte, 2017). They close their stomata relatively soon to reduce transpiration and conserve water in their tissue (Balasimha, 1988). But under drought conditions, cocoa trees allow their leaf water potential to decrease to a certain extent (dos Santos et al., 2014; Osorio Zambrano et al., 2021) indicating a partial departure from strict isohydric behaviour. This flexibility can be advantageous for maximizing photosynthesis, water-use efficiency and adjusting water status in response to environmental conditions (de Almeida et al., 2016; Daymond et al., 2011; Lahive et al., 2019).

1.7 The role of potassium

Potassium (K) plays a crucial role in various physiological processes vital to growth, yield, and stress tolerance of all crops (Marschner, 1995). It is highly mobile and taken up in large amounts by plants. K plays an integral role in plant-water relationships, and is involved in numerous physiological functions where plant water status is essential such as photosynthesis, guard cell turgor control linked stomatal opening and closure, assimilate translocation (Tsonev et al., 2011), water conductance and transpiration (Arquero et al., 2006), root hydraulics (El-Mesbahi et al., 2012) and sap flow regulation (Oddo et al., 2011). In addition, K application enhances leaf water potential (Levi et al., 2011), root growth and stem elongation under drought stress. The process of plant nutrition involves the absorption of various essential nutrients simultaneously from the soil solution through their root system based on their availability and the plant's needs, and K is indeed one of these critical nutrients. K itself directly intervenes in transport of sugars from leaves to fruit and other parts of the plant (Marschner, 1995). K application to crops could thus potentially be an effective measure to mitigate negative effects of drought.

Indeed, K application has been shown to mitigate the negative effects of drought on leaf water potential, leaf starch content, sugar partitioning and yield in several crops such as highland banana (Taulya, 2013), olive (Erel et al., 2014) and cassava (Ezui et al., 2017). This makes it relevant to explore to which extent K application may mitigate drought in other perennial crops facing similar challenges, such as cocoa. However, it is essential to note that most African soils have low fertility and are poor in K (Wang et al., 2013).

In the case of cocoa cultivation, K application has been considered as a potential mitigating agent of the negative effect of drought in the crop stands (Medina & Laliberte, 2017). Cocoa pods are very rich in K and large amounts are exported from the field during harvest, which emphasizes the need for proper K nutrition in the crop.

This requirement is often not met due to low soil K availability resulting in yield reduction. Cocoa studies on seedlings indeed suggests that K application can mitigate drought stress (Djan et al., 2018; Kaba et al., 2022), but it is unknown whether this also holds for mature field-grown cocoa trees.

1.8 From cocoa leaf level physiological processes to yield

Physiological processes are often measured at organ level and quite commonly at the leaf-level. But crop performance such as harvestable yield is measured at the crop stand (plant population) level. The way in which individual leaf responses to drought affect the canopy processes and crop yield is complex. Canopy photosynthesis, for instance can be viewed as the product of light capture and photosynthetic light-use efficiency also known as the canopy light-use efficiency (LUE). Limited soil water availability often leads to reduced leaf area either through enhanced senescence or reduced leaf production, diminishing light capture and active photosynthetic area, thus impacting growth and yield. This adaptive strategy minimizes water loss through transpiration but thus also comes at a cost to the plant as it reduces light capture (de Almeida & Valle 2007; Carr & Lockwood, 2011; dos Santos et al. 2014; Kunikullaya et al., 2018). While fewer leaves may optimize water use, they result in direct losses in canopy photosynthesis.

Simultaneously, lower stomatal conductance (Gs), a typical drought response, restricts CO_2 entry and directly reduces photosynthetic rates, negatively affecting canopy LUE (Jin et al., 2023). The combined effects of leaf area reduction and changes in Gs add complexity to understanding plant physiology, particularly in cocoa with its flushing behavior, where leaves are produced in sudden burst of leaf formation several times a year (Alvim et al., 1977; Zuidema et al., 2005). Understanding this dynamic is essential for a comprehensive view of the impact of drought on canopy photosynthesis and plant productivity.

In turn, scaling up from leaf and canopy photosynthesis to yield is complex in cocoa, where beans are the harvestable product. That because the yield of a cocoa plant is measured by the quantity and quality of the cocoa beans it produces, not just the biomass or the number of cocoa pods. Limited water and nutrients can reduce yield by affecting CO_2 absorption or nutrient uptake, or by altering resource allocation. For example, drought may lead trees to prioritize root growth over pod development (Rengel et al., 2022), further impacting yield. Reproductive factors like flowering and pod set also play a role, as they are influenced by resource availability (Farooq et al., 2009; Carr & Lockwood, 2011; Gateau-Rey et al., 2018). Drought not only directly affects these processes by limiting resources for pod development but may also indirectly impact factors like pollinator availability or flower pollination success.

1.9 Plant adaptation

The environmental conditions in which plants live are at least to some extent variable. Plants being sessile cannot move away and therefore need to be able to deal with these fluctuations (Schulze et al., 2002). Indeed, plants have different ways of adjusting their characteristics to deal with these conditions by adjusting their functioning through several processes collectively known as adaptation (Alscher & Cumming, 1991). Adaptation to a new environment comprise genetic adaptation (responses occurring over generations by genetic/inherited changes) and phenotypic adaptation typically referred to as acclimation and/or plasticity in the performance or traits of individuals in response to changing conditions during their lives. The distinction between these two is somewhat arbitrary but ‘acclimation’ typically refers to physiological changes and has been considered to be a special case of plasticity (Laland et al., 2014; Laland et al., 2015). Acclimatory responses are often reversible. For example, plant leaves may change color, through alteration in the amount and composition of pigments in response to changes in light conditions (Savolainen et al., 2004; Chevin et al., 2013; Franks et al., 2014). The earlier mentioned changes in stomatal opening also represent a fast and fully reversible phenotypic response. Other phenotypic changes can be slower (e.g. occurring over periods of days, weeks or even months) and less readily reversible. Such responses include changes in biomass allocation or changes in fruit wilt.

Genotypic changes, on the other hand, refer to alterations in plant genetic DNA sequences which typically occur due to mutations. These changes can be passed on to offspring through reproduction and are generally not reversible (Falconer, 1952; Athanasiou et al., 2010; Murren et al., 2014; Murren et al., 2015). In the case of responses to low water availability, adaptation strategies in plants may reduce the negative impacts of drought stress (Blum, 2005; Blum, 2011). To select drought-tolerant crop genotypes, performance of various genotypes can be evaluated under different drought scenarios (Medina et al., 2017; Dzandu et al., 2021).

In this regard, large variation in genotypic and phenotypic responses to low soil moisture have been reported already in cocoa. Genotypic differences in several morpho-physiological responses were found in cocoa clones and hybrid varieties (dos Santos et al., 2014; Alban et al., 2016). Studies reported that thick leaves, efficient stomatal closure, photosynthesis and high tissue elasticity were the traits expressing adaptation of cocoa plants to drought (Apshara & Krithika, 2018). Certain cocoa genotypes were better equipped to withstand drought stress exhibiting adaptive mechanisms that allowed them to maintain essential physiological processes than others that were more susceptible (Wiredu et al., 2011; Medina & Laliberte, 2017; dos Santos et al., 2018; Sauvadet et al., 2021).

1.10 Drought mitigation through agronomic practices

Farmers can mitigate the negative effects of drought through adaptive agronomic management practices helping adaptation processes in plants. Such practices are applied to attain better crop productivity through more efficient use of agricultural inputs in this case especially water (Parry et al., 2005). Efficient agronomic practices need to be tailored to suit specific drought scenarios and optimize crop resilience (Antwi-Agyei et al., 2012; de Souza et al., 2015; Díaz-José et al., 2016).

This thesis focuses on two main drought-related agronomic practices: irrigation and nutrient management. Irrigation, as a direct application of water to the soil, is the most straight forward practice for mitigating drought, ensuring optimal crop growth and production (Koech & Langat, 2018). The timing of irrigation is critical, considering both the dynamics of water availability and the developmental stage of the crop (Carr & Lockwood, 2011; Agele et al., 2016; Olamide et al., 2022). Effective irrigation scheduling involves monitoring soil moisture and evaporation. However, challenges such as installation and maintenance costs, along with potential competition for water resources, particularly in areas with multiple crop demands, need careful consideration (Davis & Dukes, 2010; Olamide et al., 2022). While irrigation has been studied in the context of cocoa seedlings (Hutcheon et al., 1973; Ahenkorah et al., 1974; Jadin & Jacquemart, 1978; Lahive et al., 2019; Dincher et al., 2022), research on its effects on mature cocoa physiology and yield under field conditions is limited (Mensah et al., 2023).

Another practice that could serve to mitigate drought effects is the application of K fertilizer (Maschner, 2011), as discussed in the section "*The role of potassium*". Fertilization in cocoa cultivation typically aims to compensate for nutrient exports due to pod harvests or address deficiencies and imbalances in soil nutrients (Koko et al., 2011; Kassin et al., 2016; Snoeck et al., 2016). However, the extent of K application in cocoa cultivation varies based on recommended fertilizer formulas, which may not account for regional differences in soil conditions and yield levels. This lack of considering local conditions may hinder sustainable cocoa production. Some countries recommend a single fertilizer formula, while others have adopted new formulations with alternative compositions (Koko et al., 2011).

1.11 Research Aim of this Thesis

This thesis presents the results and analyses of two contrasting experiments conducted in two different agro-ecological areas, and quantifies the effects of water deficit and K application on the physiology, growth and productivity of cocoa trees.

The first experiment was conducted in a wet area (1,600 – 2,200 mm) where the severity of drought effects was studied through rainfall reduction, simulating the effects of reduced rainfall during the wet season. The idea behind this experiment was to explore how potential reductions in rainfall may affect crop performance and yield. The second experiment was performed in a drier agro-ecological area, where the effects of dry periods on drought-tolerant varieties were either or not alleviated using irrigation as a mitigation strategy. In the latter experiment, I compared different cocoa genotypes. In both experiments different levels of K fertilizer were applied. Using these experiments, I explored the combined effects of soil water availability and K fertilizer on different cocoa genotypes, spanning physiology, growth, reproductive phenology, and yield.

Three main research questions were asked:

1- How does experimentally-induced water deficit (Chapter 2) affect the physiology and overall performance of cocoa trees, and can K application mitigate the negative effects of such drought conditions?

2- In what ways can the physiological stress induced by limited water availability for cocoa trees be alleviated through irrigation, and how does this interact with K application to enhance tree resilience (Chapters 2 and 3)?

3- How do annual cocoa yield and its reproductive components vary among different genotypes in response to soil water deficit and K application (Chapter 3 and 4)?

1.12 Study area

Côte d'Ivoire, situated in the intertropical area, exhibits diverse climates, including a moist tropical climate in the south and a sub-humid savannah climate in the north. Temperatures across the country average between 25 to 27 °C. Annual rainfall varies from 1,000 to 1,600 mm, with higher amounts in the south and lower in the north and centre. The south-western evergreen forests receive up to 2,200 mm of rainfall annually. This study explored two distinct agro-ecological areas (AEZs) in Côte d'Ivoire (Aregheore, 2009). The southern forest area (AEZ I) is characterized by high temperatures around 30°C and heavy rainfall between 1,200 and 2,400 mm, experiencing a long rainy season (April-July), a short dry season (August-September), a short rainy season (October-November), and a long dry season (December-May). In contrast, the northern savannah area (AEZ II) features a sub-humid climate with temperatures of 28 to 37°C and annual rainfall of 400 to 1,200 mm. Its four seasons include a long rainy season (April-mid July), a short dry season (mid-July-September), a short rainy season (September-November), and a long dry season (December-March) (Bationo et al., 2006; Dekoula et al., 2018; Gnangui et al., 2021). These variations in climate present unique challenges and opportunities for agriculture (Aregheore, 2009). Consequently, Chapter 2 of this thesis focuses on experiments in the Divo region (AEZ I; forest area, Figure 1.1), while Chapters 3 and 4 are based on studies in the Zambakro region (AEZ II; savannah area, Figure 1.1).

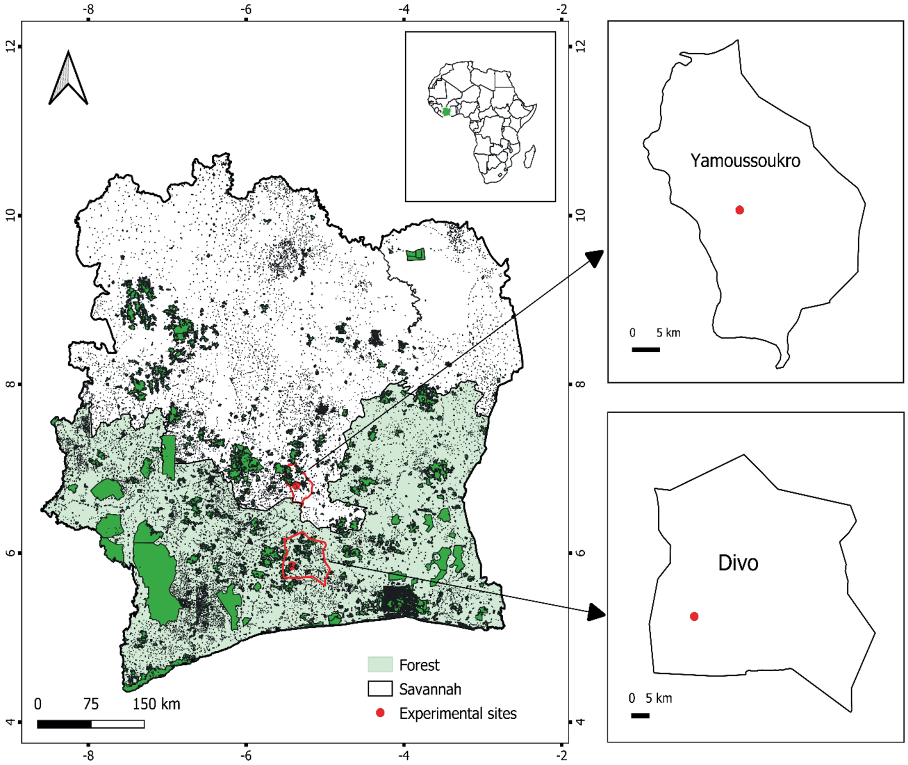

Fig. 1.1. A map of Côte d'Ivoire delineating the Forest and Savannah agro-ecological areas, featuring the two study areas in red color. The lower and upper graphs in the right panels provide an overview of the individual study areas: Yamoussoukro where the Nestlé R&D centre was located and the CNRA centre in Divo (Adet, 2024).

1.13 Outline of the thesis

This thesis is organized in five chapters. In these chapters, the physiological responses at leaf and tree level, the yield responses and tree performance under water deficit and K application are analyzed and discussed (Fig. 1.2). To my knowledge no study has assessed these interactive effects yet in fully mature cocoa trees. According to Lahive et al. (2019), one of the main research gap that needs to be investigated is the effect of the interaction between drought and K on cocoa responses.

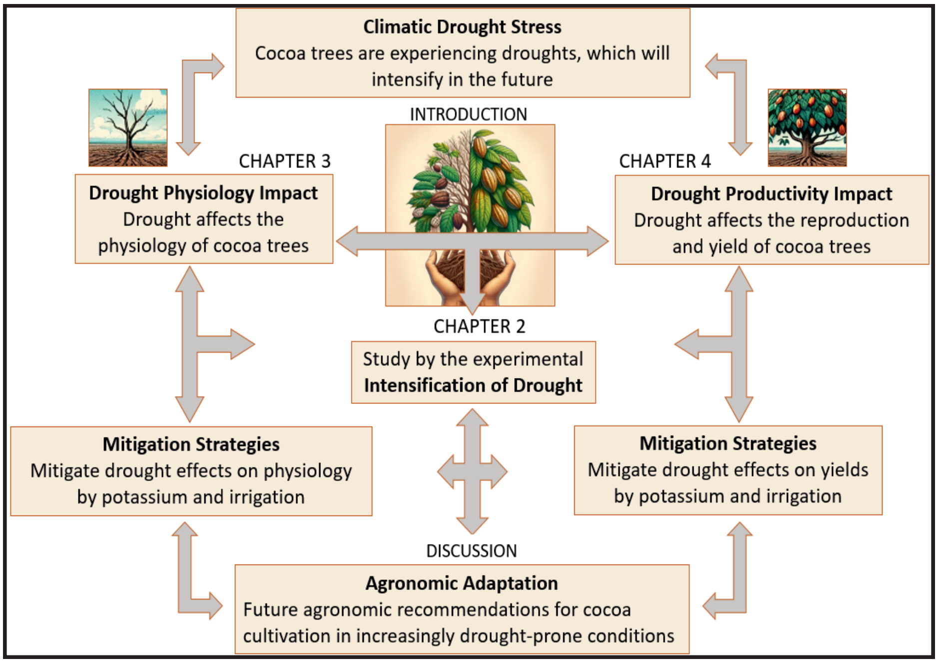

Fig. 1.2. Contrasting approaches assessing and mitigating drought Effects. This figure illustrates the research framework, which involves two contrasting approaches. The first approach assesses the effect of rainfall reduction, highlighting the impact on overall cocoa tree performance (Chapter 2). The second approach investigates strategy for mitigating the negative effect of water deficit, emphasizing the role of irrigation supply on the cocoa physiological responses (Chapter 3) and overall productivity of different drought-tolerant varieties (Chapter 4).

Chapter 1: General Introduction.

This Chapter introduces the background and sets the context for this thesis in terms of research problem, questions, and targeted research areas. It includes a general overview about what **soil water deficit** means **for plants** specifically for **cocoa** and also the pathway **from physiology processes to yield** and the main **agronomic practices** helping plants to tolerate drought stress.

Chapter 2: Cocoa trees performance and yield are affected by seasonal rainfall reduction.

This chapter assesses the possible consequences of drier conditions due less water reaching the soil during the rainy seasons in combination with K application on cocoa tree general performance, referring to the thesis research question 1. It is thus linked to the question: what if rainfall is reduced in the future?

Chapter 3: Genotypic differences in water deficit effects on leaf and crown traits in mature field-grown cocoa

This chapter investigates the effects of water deficit, K application and their interaction on leaf physiology of mature field-grown trees of six cocoa genotypes to answer the thesis research question 2. It is thus linked to the question how different strategies could mitigate drought effects.

Chapter 4: Negative effects of water deficit on cocoa tree yield are partially mitigated by irrigation and potassium application

It addresses the thesis research question 3 to answer how does annual cocoa yield come to be, focusing on the response of various parameters that explain yield in condition of soil water and K availability.

Chapter 5: General discussion.

This Chapter presents a general discussion, overview of all the results presented in Chapters 2, 3, 4 and the derived conclusions from each result related to the initial objectives and research hypotheses and ends with practical recommendations and suggestions for further studies

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Chapter 2

Cocoa tree performance and yield are affected by seasonal rainfall reduction

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This Chapter has been submitted for publication

Abstract

In West Africa, long dry spells are likely to become more frequent and intense as a result of global climate variability, which may significantly impact cocoa tree performance (i.e., morphology, physiology, growth and production). The application of potassium (K) may mitigate the negative drought impacts on cocoa functioning and yield. However, limited knowledge exists on how the reducing duration of the wet season and its drier characteristics affect cocoa functioning and yield, and whether effects can be mitigated by K availability. The aim of this study was to investigate the effects of reduced water availability via shelters and K application on cocoa leaf traits, root growth, reproductive dynamics and yield of cocoa trees in a 6-year old plantation. Two soil moisture levels and two K treatments were considered: a control (no shelter) and sheltered (67% rainfall reduction) treatment, either with or without 200 kg/ha K application. Our results showed that the reduction in rainfall significantly decreased soil moisture by 9.1%, total root length by ~57% and root mass density by ~50%. It also reduced stomatal conductance (Gs) by ~60%, leaf flush intensity by ~70%, leaf greenness ~48% and leaf size by ~68%. Water availability reduction did not influence individual pod mass, but significantly reduced flower intensity, production of healthy cherelles, pod and bean numbers per tree. Together this resulted in a dry bean yield reduction from ~2100 kg/ha to ~1450 kg/ha (i.e., about 31%). The beneficial impact of K application was primarily noticeable under control conditions, where it increased Gs, leaf size and greenness. Our results indicate a strong negative effect of reducing water availability on cocoa yield, and that this response is mediated at various levels from leaf physiology to pod production However, our results suggest that potassium application may not mitigate drought effects. Climate smart agricultural practices that combine precision irrigation, nutrient management and improved crop varieties (hybrids) are needed to sustain cocoa growth and yield under increasing variability in rainfall.

Keywords: Cocoa trees, dry seasons, physiology, yield, water dynamics

2.1 Introduction

Cocoa is of great importance for the livelihood of millions of smallholder farmers in West Africa as it serves as a major source of income, contributing to poverty reduction and economic growth (Peprah, 2015; Olwig et al., 2024). Cocoa farming creates economic opportunities not only for farmers but also for various actors along the cocoa value chain (Laven & Boomsma, 2012). However, cocoa cultivation is confronted to significant challenges resulting from the potential adverse effects of climate change, resulting in warming and in less regular rainfall (Schroth et al., 2016). Climate change models predicted declines in rainfall for parts of the West African cocoa belt, especially in Ivory coast and Ghana (Asante, 2023). Many cocoa-growing areas will face increasingly severe dry seasons (Dai, 2011; Abdulai et al., 2018; Ajayi & Ilori, 2020) as a result of reduced rainfall. In recent years, shifts in the timing of the wet and dry seasons have negatively affected cocoa production. These shifts in rainfall distribution will likely result in a decline in the suitable areas for cocoa cultivation (Abdulai et al, 2018; Bunn et al., 2019), threatening farmers' livelihoods and the sustainability of the chocolate industry (Ruf & Schroth, 2004; Peprah, 2015; Asante et al., 2017).

Cocoa trees are particularly sensitive to variations in rainfall which can lead to reduced tree performance and yield, and increased vulnerability to diseases and pests (de Almeida et al., 2016; Anning et al., 2022; Mensah et al., 2023). Several studies have examined how variation in water availability impacts cocoa crops. Specifically, these studies have investigated the impact of adding water through irrigation as a potential drought mitigation strategy (Hutcheon et al., 1973; Adet et al., 2024). However, few studies have explored the effects of reduced rainfall. There is a distinction between irrigation experiments and rainfall reduction experiments in the way water availability is manipulated. Irrigation experiments involve adding water and hence mitigating drought stress without changing ambient, stressful air conditions, while rainfall reduction experiments apply shelters to reduce the part of the rainfall that reaches the soil to intensify drought stress (Dai, 2011). In addition, the timing of the two interventions can also be different. Irrigation is often applied during the dry season

while sheltering tends to be done during the wet season. This may cause results of these two types of experiments to vary, because leaf and reproductive phenology of cocoa trees differs between dry and wet seasons.
Cocoa trees exhibit an array of changes in their physiological and morphological traits in response to changes in soil water availability (Zuidema et al., 2005; de Almeida & Valle, 2007; Osorio Zambrano et al., 2021). A reduction in rainfall was found to limit fine root growth in cocoa trees restricting their ability to access water and nutrients in dry periods (Kummerow et al., 1982; Mommer, 1999; Nygren et al., 2013; Moser et al., 2010; Niether et al., 2019). Moreover, reduced rainfall triggered a decrease in stomatal conductance (Gs), leading to reduced leaf photosynthesis (Adet et al., 2024). Additionally, cocoa trees experience reductions in leaf greenness, specific leaf area, leaf area expansion, and flush intensity in response to drought (Tezara et al., 2016; Rao et al., 2016). Furthermore, rainfall reduction negatively impacted various aspects of cocoa reproduction, including flowering, pod development, and cherelle and pod wilting. When rainfall reduction coincides with crucial reproductive stages, it can lead to a decrease in the number of pods produced, in pod size, and a decline in bean quality resulting in a diminished cocoa yield (Aikpokpodion et al., 2003; Omotayo et al., 2018).

Potassium (K) plays a major role in regulating physiological processes in plants, and these responses have been shown to increase tolerance to drought stress (Wang et al., 2013). It is also an indispensable nutrient for cocoa, among others because it is removed from cocoa plantations by harvesting pods (van Vliet et al., 2015). K application has been proposed as a promising strategy to mitigate the adverse effects of drought on cocoa growth (de Almeida & Valle, 2007; Djan et al., 2018). Indeed, adequate K nutrition contributed to improved water-use efficiency, enhanced photosynthesis, and greater stress tolerance in cocoa seedlings (Kaba et al., 2022). In others crops such as cassava (Chua et al., 2020) and banana (Taulya, 2013), K application has also been shown to mitigate negative effects of drought on crop yield. However, whether these findings also hold for mature trees under field conditions is unknown (Lahive et al., 2019). A recent study showed that in mature cocoa K application did not mitigate negative drought effects on leaf water potential, Gs, and leaf area (Adet et al., 2024).

In this study, we assessed the effects of reduced rainfall through rainfall interception and K application individually or interactively on (1) cocoa root traits, (2) leaf physiology and morphology traits, and (3) reproductive dynamics and yield. To this end, shelters were used during the rainy season to reduce the amount of rainfall reaching the ground (throughfall and stemflow) under the cocoa trees, and trees were provided either with K fertilizer or not. We hypothesized that reduced rainfall will: (i) decrease root mass density; (ii) lead to lower Gs, lesser leaf greenness, smaller leaf area, smaller specific leaf area, and lower flush intensity; and (iii) negatively affect flower intensity and cherelle production, as well as pod, bean production and annual yield. We expect that K application will mitigate the effects of water stress.

2.2 Material and methods

2.2.1 Study area

The experiment was performed at the "Centre National de Recherche Agronomique" (CNRA) research station, situated in Divo, Côte d'Ivoire (5°48' N, 5°18' W) from April 2020 to April 2022. Divo is situated in a forested area of Côte d'Ivoire, known for its favorable climatic conditions for cocoa production (warm temperatures, adequate rainfall, and high humidity). The area has an average annual temperature ranging from 24 – 26.7˚C with an average rainfall of 1200 mm per year. Rainfall has a bimodal distribution normally with a long wet season from mid-March till late June, a short dry season in July and August, a short wet season from September till November and a major dry season from December to early March (Tosto et al., 2022; Tosto et al., 2022). However, the rainfall distribution can vary between years.

The experimental design was a randomized block design with six replicates (Fig. A.2.4). The plot consisted of six rows of 24 cocoa trees with two border rows on each side. Within each experimental unit, measurements were conducted on the four central trees. The experiment utilized the 'Mercedes' cocoa hybrid variety, widely used and an improved variety in Côte d’Ivoire known for its high yields (Eskes, 2011) and relative drought tolerance (personal observation). Mature six-year-old trees, arranged in a 3 x 2.5 m planting design (~1320 plants/ha), were used, with half of the plot subjected to a rainfall reduction treatment under shelter.

All the trees received 156 kg/ha/year of Di-Ammonium Phosphate (DAP) and 487.5 kg/ha/year of Nitrabor; in total, which amounted to 174 kg N/ha and 72 kg P/ha. Nitrabor not only provides 15.4% nitrogen (N), 14.1% Nitrate (NO3), 1.3% Ammonium (NH4) but also includes 25.9% calcium oxide (CaO) and 0.3% boron (B). The potassium application treatment was randomly assigned to the experimental units.
Potassium application was applied using potassium chloride (KCl) at rates of 0 and 206.7 kg per hectare per year, corresponding to 0 and 124 kg of K per hectare. These treatments are referred to as 'without K' and 'with K,' respectively. The fertilizers were applied at a distance of 40 cm around each tree into three equal applications in April, July, and September. The first dose of fertilizer was applied prior to the start of the rainfall reduction treatment in May to provide the necessary nutrients at the onset of the induced dry months. The border tree lines were included to prevent mutual interference between plots and treatments, ensuring that potential effects or interactions between adjacent plots are minimized.

2.2.2 Rainfall reduction treatment

The rainfall reduction treatment reduced the amount of direct rainfall to experimental plots to investigate its effects on cocoa tree responses. To this end, we used shelters of transparent plastic to intercept and decrease the rainfall reaching the ground to mimic water deficit conditions. They were positioned in the middle of the month with distinctly higher rainfall during each wet season. During initial installation, sheltering was delayed by two months. The shelters were removed when the rains stopped. Shelters were installed for 14 months (May – Jul and Sep – Nov 2020; Jan – April and Aug – Sep 2021 and Mar – Apr 2022). Nine of these received over 100 mm of rainfall and five having less than 100 mm (see Fig. A.2.1). The months during which the shelters were installed varied across years due to differences in the timing of rainfall.
Plastic shelter bands with a width of 2 m were used to cover the soil surface during the abovementioned months. These bands were placed horizontally between tree rows (12

in total) and intercepted 67% of the throughfall during the 14 months when shelters were on the field (Table A.2.2).

The rainwater, intercepted by the plastic bands, was diverted to trenches outside the experimental plot, at the lower side of the slope to prevent water reflux. The volumetric soil water content was measured monthly at 20 cm depth throughout the experiment at the base of all selected sheltered and unsheltered trees, using a FieldScout TDR 350 handheld device.

2.2.3 Rainfall partitioning

We partitioned rainfall into its components: the part that penetrates the canopy directly (throughfall), that runs along the stem (stemflow), and the part that was intercepted by the canopy and then evaporated without reaching the soil (interception loss). Interception loss is defined as the portion of rainwater that is retained or intercepted by vegetation or litter above the ground with only part of it reaching the ground as throughfall (Ward & Robinson, 2000). This intercepted water contributes to the total amount of evapotranspiration. In our study, data on stemflow and throughfall were collected from 42 sheltered trees during the months when the shelters were in place, from which the interception loss was subsequently calculated by subtracting the sum of stemflow and throughfall from the rainfall. To quantify throughfall, water was collected in the plots where shelters had been placed, using 5 litre-capacity polyethylene bottles with an opening of 95 cm^2 to capture the rainfall that passed through the trees' crown. Seventy-two bottles were installed beneath the canopy at regular intervals, at 0.5 m, 1.5 m and 2.5 m, from the trees in a straight line within each experimental unit (Mensah et al., 2023). The bottles were put at 0.25 m above the ground to prevent water droplets and soil particles splashing in. Stemflow water was collected using plastic petticoat-type gauges wrapped around the trees, as a conduit to channel the rainfall from the trunk to the 25 litres collecting tank. Stemflow was converted from liters per tree (L $tree^{-1}$) to mm (i.e., liters m^{-2}) by taking the projected crown area of the trees, which we assumed to be equal to the area available to each tree which when trees are spaced 3 x 2.5 m is 7.5 m^2. The formulas used to derive interception loss and water availability are included in Supplementary Material Table A2.2.

2.2.4 Root measurements

Three soil cores per tree were extracted to measure root mass density in a cylinder volume of 441 cm^3. The roots were separated from the soil with a 2 mm sieve while rinsing with running water, followed by a wash with distilled water to eliminate any residual debris. After washing, the roots were directly weighed to obtain their fresh mass per unit of soil volume and then placed in an oven at 70 °C for a period of 48 hours. After drying, the roots were reweighed again to obtain their dry mass and calculate the root dry mass per unit of soil volume referred to as root mass density onward.

Soil trenches were dug to a depth of 60 cm to examine the root systems at different depths. A grid divided into small squares, each with an area of 25 cm^2, was affixed vertically to the trench walls and aligned perpendicular to the trees being studied (Tardieu & Manichon, 1986). While visually inspecting the soil profile within each grid square, the number of root interceptions was recorded to calculate the total root length in cm within the grid, following the equation: Total root Length = $(11/14) \times N \times 5$ cm, where N represents the number of root interceptions recorded within the grid and 5 cm is the grid size (Tennant, 1975).

2.2.5 Leaf greenness

The leaf greenness was measured using a SPAD-502 portable chlorophyll meter (Konica Minolta Inc., Tokyo, Japan), which provides a proxy for leaf chlorophyll content. Randomly selected mature and fully expanded leaves from the second-youngest flush were chosen for the measurements. The SPAD meter readings were taken at the midrib of each selected leaf on both leaf sides. Measurements were taken on five unshaded leaves, five middle canopy leaves, and five lower canopy leaves per tree in each treatment within each block. SPAD measurements were taken every month throughout the experimental period.

2.2.6 Stomatal conductance

Gas exchange measurements were performed using a portable SC-1 Steady State Leaf Porometer (Decagon Devices, Pullman, WA, United States). Stomatal conductance (Gs) was measured on the interveinal areolae at the mid-lamina of the abaxial (lower) surface of each leaf. The leaves were intact, green, healthy, mature, exposed to sunlight and without signs of disease or damage. The leaf was allowed to equilibrate for a short period of approximately 30 to 120 seconds. The reading was recorded once the equilibration period was completed. The leaf sample readings that took longer than three minutes to equilibrate were discarded. The Gs measurements were taken on the four central trees between 7:00 h and 9:30 h during both dry and wet seasons. This time window was chosen to conduct measurements before the significant decrease in relative humidity and increase in heat. In the wet season, measurements were done on days without rain.

2.2.7 Leaf size measurements

Leaves from three canopy layers (high, mid, low) were randomly sampled during each season (long/short dry/wet seasons) to measure leaf fresh weight, leaf dry weight, and leaf area. Leaf pictures or scans were first taken and then analysed using the image analysis software ImageJ to derive leaf area. Leaf fresh weight was determined, and then leaves were oven-dried at 65°C for 48 hours. The data for the four monitored trees were averaged per tree per plot. Specific leaf area (SLA) was calculated as leaf area (cm^2)/ leaf dry weight (g).

2.2.8 Monitoring cocoa pod development and yield

Four central trees within each experimental unit were selected to monitor pod development and yield during two years. The number of flowers produced in the cushions to characterize flowering dynamics was monitored monthly. A scoring system from 0 to 4 was used to rate the intensity of flowering for each tree, with 0 indicating no flowering and 4 indicating the highest level of flowering. Specifically, a score of '0' signified the absence of flowers; '1' meant that less than 25% of the tree's branches and canopy were covered in flowers; '2' indicated that flowers were present on 25-50% of the tree's branches and canopy; '3' indicated that 50-80% of the tree was covered with flowers; and '4' corresponded to a coverage of 80-100% with flowers. Cocoa pod development was monitored monthly during both minor and major harvest periods, focusing on three pod categories per tree: healthy cherelles, wilted cherelles, and ripe pods with cherelles designing the young pod in the early stages of development. The number of beans inside each sampled pod (6 pods per tree) was counted, the weight of individual sampled pods and the number of beans per pod were estimated. Finally, the annual yield per tree was calculated by multiplying the number of pods per tree with the beans per pod and the average individual bean weight (1.5 gr).

2.3 Statistical analysis

Linear mixed-effects model evaluated the effect of the rainfall reduction treatment, potassium application and their interaction (fixed effects), on cocoa root characteristics, leaf physiology, leaf morphology, production and reproduction as response variables. Additionally, we used a linear mixed-effects model analysis to evaluate the effect of the rainfall reduction treatment on the seasonal soil volumetric water content. The model included the rainfall reduction treatment, the season and their interaction as fixed effects and individual measurement locations as random effect to test whether soil water content depended on shelter treatment and/or seasonality. A random intercept was included, either per tree or per leaf (for leaf traits). For each response variable, a model comparison was performed, comparing models with all combinations of fixed effects based on the Akaike Information Criterion (AIC). Models that differed <2 AIC units were regarded to perform equally well (Akaike, 1974; Burnham & Anderson, 2002), and in the case of multiple best models, the model with the least fixed effects was selected. Bootstrapped confidence intervals were calculated to assess significance of the predictors. PostHoc tests were only performed when there was a significant interaction between shelter and potassium treatment. R-squared (R^2) values were calculated to measure how well the independent variables explain the variability of the dependent variable in a LMM model. We determined the proportion of variance explained by the fixed effects in the model R-squared marginal (R^2m) and conditional R-squared (R^2 c) accounting for both fixed and random effects in explaining the variability in the data. Statistical analyses were performed using R software version 4.2.2 (R Core Team, 2022); linear mixed-effects models were performed using the glmmTMB package (Brooks et al., 2017).

2.4 Results

2.4.1 Effects of treatments on rainfall components and soil water availability

Relatively large volumes of throughfall were observed during July and October 2020, as well as March 2021 (Fig. A.2.1. A), while significant amounts of stemflow were recorded in June and October 2020, and in March and September 2021 (Fig. A.2.1. B). On average, monthly throughfall accounted for 62% of the monthly rainfall, ranging from 22% to 91%, while stemflow accounted for 3%, ranging from 0% to 8%. Consequently, approximately (62+3=) 65% of the rainfall reached the soil, primarily through throughfall, with the remaining 35% directly evaporating from the canopy. Annual rainfall varied from 1317 mm in 2020 to 1118 mm in 2021, exhibiting notable monthly variability throughout the study period. Peak rainfall was recorded in June and October 2020, March and October 2021, and April 2022 (Fig. A.2.1. D).

In the control plots, water availability for cocoa trees was approximated as the combined total of throughfall and stemflow (Table A.2.2). Rainfall was recorded during specific monitoring periods across the experiment years. In 2020, the average rainfall during these periods was 147.2 mm per month, resulting in a rainfall accumulation of 883 mm. The year 2021 showed a decrease, with a monthly average of 102.6 mm of rainfall, and a total of 615.7 mm. In 2022, data was collected for only two months (March-April); during this period, the monthly average rainfall recorded was 105.5 mm, amounting to a total of 210.9 mm. Interception loss was significantly higher in shelter conditions compared to the control by ~36%, which reduces the amount of rainfall that reached the soil (Table A.2.1). Rainfall retained by the canopy and subsequent loss significantly influenced the amount of rain water reaching the soil, particularly during periods of low rainfall. Across all years, the values of rainfall reaching the soil, i.e water available for plant uptake, were notably lower in sheltered plots (RRS) compared to control plots (RRC). Lower RRC values were observed during May, June, and October 2020, as well as March and April 2021 indicating higher canopy interception. Conversely, higher RRS values were recorded during the same periods, indicating greater rainfall reaching the soil surface in the sheltered plots (Table A.2.1).

The measurement of rainfall distribution is important for understanding the eco-physiological impact on cocoa development. In 2020, the recorded throughfall and stemflow during monitored periods were 489.4 mm and 17.2 mm, respectively. Notably, the interception loss was significantly higher in sheltered plots at 721.5 mm, compared to 393.6 mm in control plots. This resulted in 506.6 mm of rainfall reaching the soil (RRC) in control conditions, while only 178.7 mm (RRS) did so in sheltered conditions. The following year, 2021, mirrored this pattern with total throughfall and stemflow at 445.5 mm and 23.2 mm, and interception losses at 170.2 mm for control plots versus 468.7 mm for sheltered plots. Consequently, 468.8 mm of rainfall reached the soil in control plots, in contrast to 170.2 mm in sheltered plots indicating the substantial impact of drought (shelters) on water availability to cocoa plants. In 2022, the total throughfall and stemflow were 100.8 mm and 2.5 mm respectively. Interception loss amounted to 110.2 mm for control plots, with sheltered plots incurring a greater loss of 177.6 mm. This led to a reduced RRS of 35.8 mm, compared to an RRC of 103.3 mm, further demonstrating the significant influence of shelter on water dynamics within the plots (Table A.2.1; Fig. A.2.1).

In 2020, 20% of the rainfall recorded during the monitored months reached the soil in the sheltered plots (RRS), while a significantly higher 57% was observed in the control plots (RRC). In 2021, RRS was 28% of the total monitored rainfall, indicating that slightly more rainfall reached the soil in sheltered conditions compared to 2020. The RRC for this year was higher at 76%. In 2022, only 17% of rainfall reached the sheltered soil, and 49% reached the control plots (Table A.2.1).

The shelter treatment decreased soil volumetric water content in both dry and wet seasons (Table 2.1, Fig. A.2.1. C; Fig. A.2.2). During the wet seasons, average soil water content was 20.0% in the sheltered plot, compared to 29.1% in the control plot. During the dry seasons, the sheltered plot maintained an average soil water content of 16.7%, while the control plot exhibited a much higher average soil water content of 25.7% (Fig. A.2.1. C; Fig. A.2.2).

Table 2.1: Results of mixed-effect models that assessed effects of rainfall reduction (Rain), potassium application (Pot) and their interaction (indicated with ":") on belowground characteristics (for soil water content, the effect of season was evaluated instead of potassium application), leaf physiology, production and reproduction. ** indicates significant predictors. The marginal (R^2m) and conditional (R^2c) R squared are indicated.

	Variables	**Units**	**Predictors**			**R^2m**	**R^2c**
Belowground	Soil Volumetric Water Content	%	Rain**	Seas**	_	0.20	0.32
	Root mass Density	g cm^{-3}	Rain**	Pot**	Rain:Pot**	0.05	0.07
	Total root Length	cm	Rain**	_	_	0.02	0.61
Leaf Physiology	Stomatal Conductance	mmol m^{-2} s^{-1}	Rain**	Pot**	_	0.17	0.32
	Flush Intensity	_	Rain**	_	Rain:Pot	0.26	0.33
	Leaf Greenness	_	Rain**	Pot**	Rain:Pot**	0.30	0.35
	Leaf Area	cm^2	Rain**	Pot**	Rain:Pot**	0.37	0.42
	Specific Leaf Area	cm^2 g^{-1}	Rain**	Pot	Rain:Pot	0.19	0.24
Reproduction	Flowering Intensity	_	Rain**	Pot**	Rain:Pot**	0.03	0.16
	Healthy Cherelles	_	Rain**	Pot**	_	0.01	0.16
	Wilted Cherelles	_	Rain	_	Rain:Pot	0.005	0.19
Production	Annual Yield	kg ha^{-1}	Rain**	Pot**	Rain:Pot	0.11	0.31
	Pod Number	_	Rain**	Pot	_	0.03	0.18
	Pod Mass	g	Rain	_	_	0.01	0.12
	Pod Bean Number	_	Rain **	Pot**	Rain:Pot**	0.21	0.44

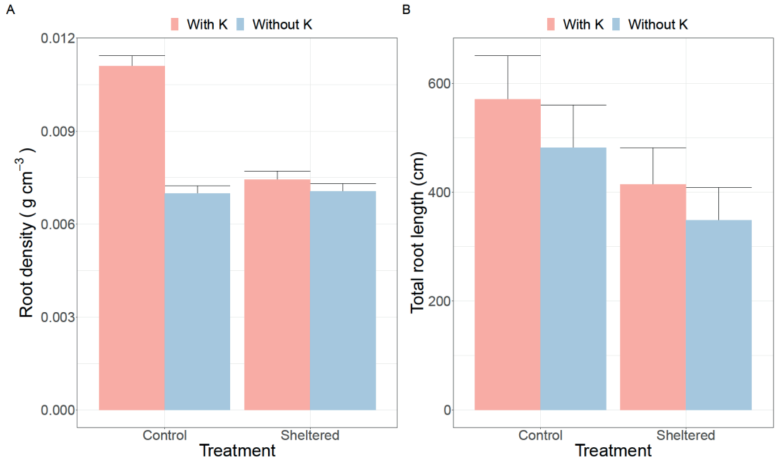

Fig. 2.1. Effects of shelters (control vs sheltered) and potassium application (With K vs Without K) on root response variables. A- root density referring to root mass per unit soil volume. B- Total root length (Control vs Sheltered) and potassium availability (With K vs Without K). Values represent means ± standard error. Different letters above the bars indicate a significant difference.

2.4.2 Treatment effects on cocoa tree functioning

There was a significant shelter by K interactive effect on root dry mass density (Table 2.1). Unexpectedly, control trees exhibited a considerably greater root mass density compared to sheltered trees where the roots were reduced by ~50 %, but only when K was applied (Fig. 2.1. A). In this case, the trees may have responded to K application by adjusting their root development in a way that was not solely dependent on rainfall. Control trees also had significantly higher total root length compared to the sheltered trees, but the effect of K was not significant (Table 2.1; Fig. 2.1. B). The negative effect of the sheltering treatment on root length density was most apparent in the shallow soil layers, from 0-10, 10-20, and 20-30 cm depth, as the root density per 25 cm^2 in the control trees was greater than in the sheltered trees. This difference disappeared in deeper soil layers from 30-40, 40-50, and 50-60 cm depth (Fig. A.2.3).

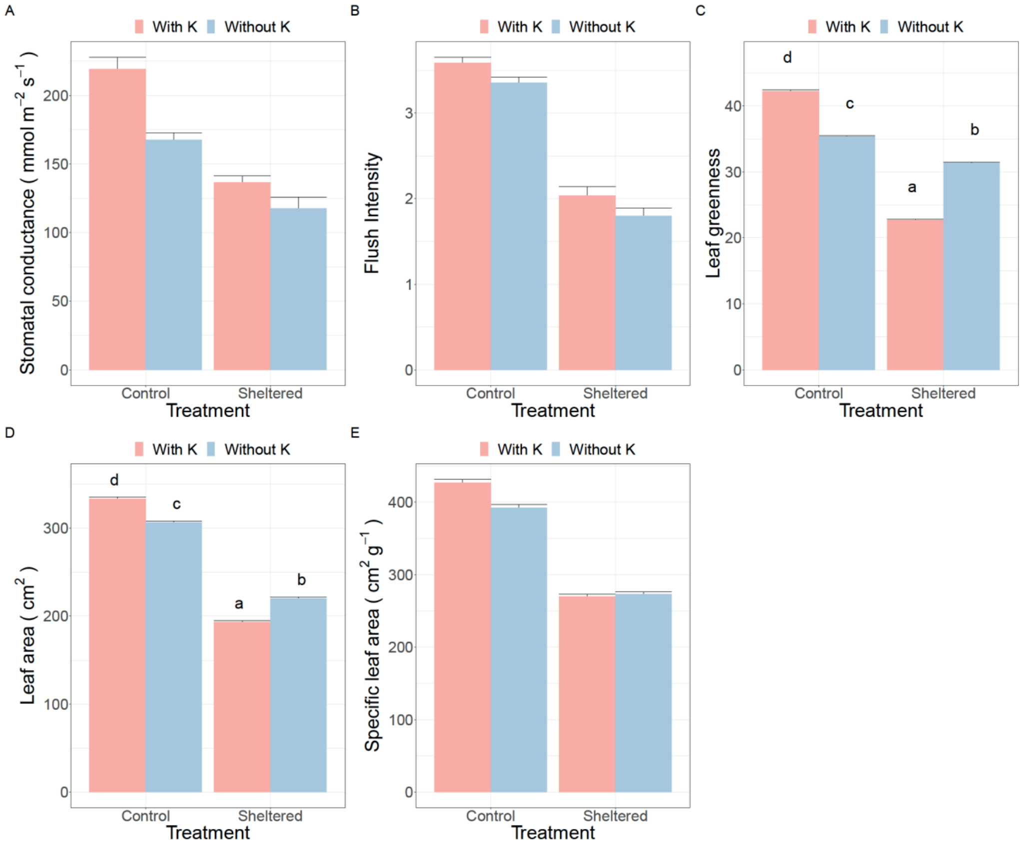

Fig. 2.2. Effects of shelters (control vs sheltered) and potassium application (With K vs Without K) on leaf traits: A- stomatal conductance, B- flush intensity scores (the number of new flushes), C- leaf greenness (SPAD values), D- leaf area and E- specific leaf area. Values represent means ± standard error. Different letters above the bars indicate a significant difference.

The shelter treatment and K application had a significant effect on cocoa leaf physiology and morphology (Table 2.1). Sheltering significantly reduced Gs by ~60% while K application positively affected Gs. The Gs response to K tended to be stronger in the control trees compared to sheltered trees (Fig. 2.2. A), but the K x shelter interaction was not significant (Table 2.1). Flushing intensity was significantly and negatively influenced by the shelter treatment (Table 2.1; Fig. 2.2. B), but K did not significantly influence flushing intensity (Table 2.1). Cocoa leaf greenness was significantly affected by the shelter treatment, K application and their interaction (Table 2.1). Leaf greenness was lowered by ~48% in the sheltered than in control trees. When K was applied to the control trees, leaf greenness increased (Fig. 2.2. C). However, K appeared to have a negative effect in the sheltered treatment. There were significant effects of the shelter treatment, K application, as well as their interaction on individual leaf area (Table 2.1; Fig. 2.2. D). The presence of the shelters had a negative effect on leaf area, indicating that cocoa trees under sheltered conditions had smaller leaves (~68% decrease). The K effect was small, with an increase in leaf area in control trees and a decrease in sheltered trees (Fig. 2.2. D). The shelter treatment significantly and negatively affected the specific leaf area (SLA), with lower SLA for sheltered trees and no significant effect of K (Table 2.1; Fig. 2.2. E).

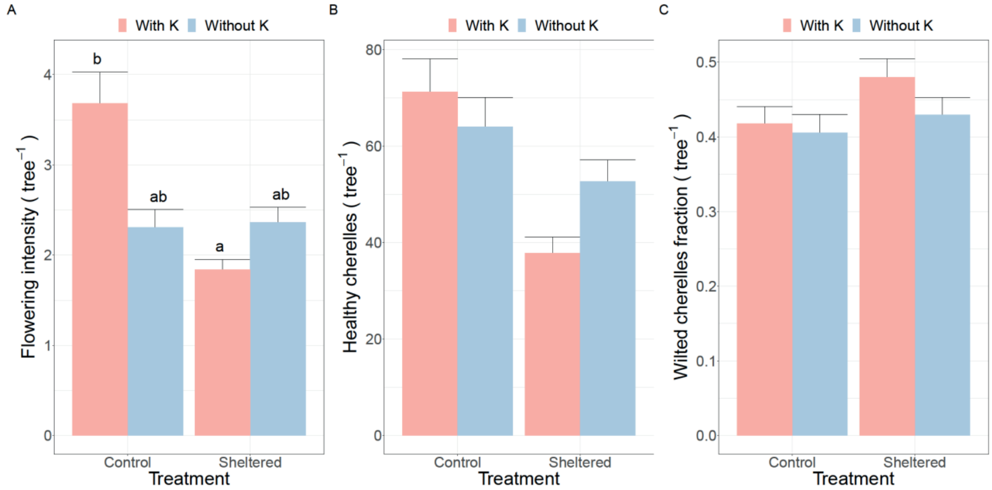

Fig. 2.3. Effects of shelters (control vs sheltered) and potassium application (With K vs Without K) on reproduction. A- flowering intensity score per tree (mean score for the number of flower cushions/tree). B- average number of healthy cherelles produced per tree. C- the fraction of wilted cherelles per tree. Values represent means ± standard error. Different letters above the bars indicate a significant difference.

2.4.3 Treatment effects on cocoa reproduction and yield

The shelter treatment and K application also had an effect on reproduction (Fig. 2.3, Table 2.1). Flowering intensity, as indicated by 5 graded scores for the number of cushions with flowers per tree, was significantly influenced by the shelter treatment, K application, and their interaction. Trees in the control treatment produced more flowers (~65% higher score) than sheltered trees, when not fertilized with potassium (Fig. 2.3. A). The shelter treatment and K application significantly affected the number of healthy cherelles (Table 2.1). Control trees had a higher number of healthy cherelles (~55%) than sheltered trees (Fig. 2.3. B). Interestingly, K application tended to have a negative effect on the number of healthy cherelles in the sheltered trees, but a weak, positive effect in the control trees. There was no effect of either rainfall reduction or K application on the occurrence of cherelle wilt (Table 2.1; Fig. 2.3. C).

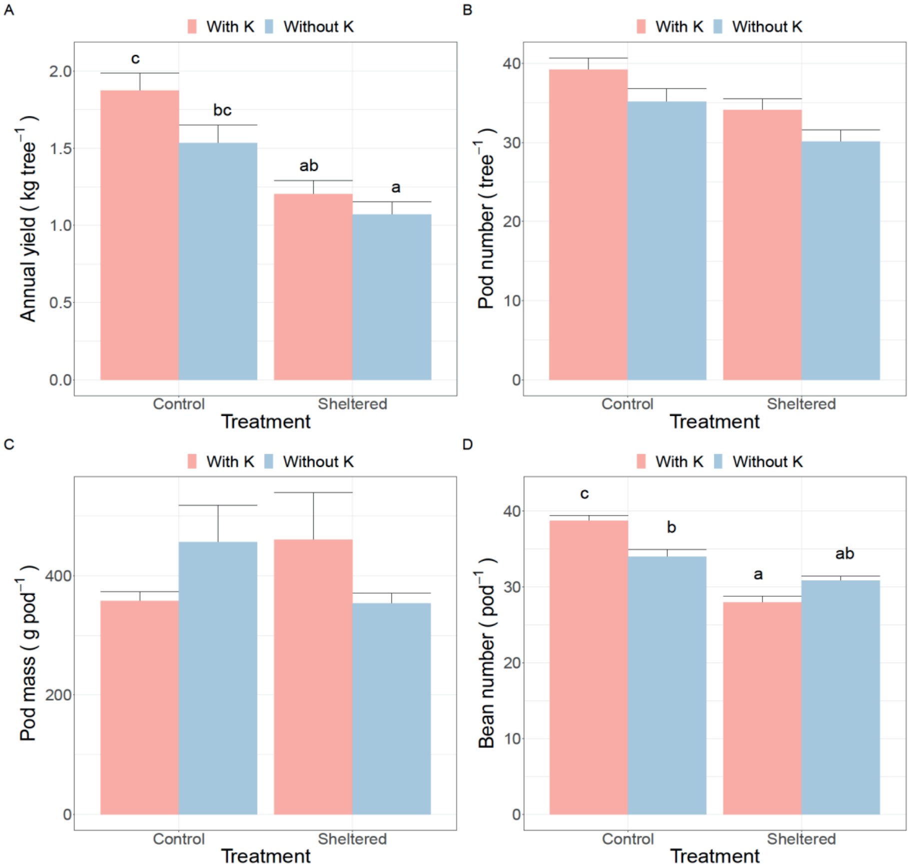

Fig. 2.4. Effects of shelters (control vs sheltered) and potassium application (With K vs Without K) on cocoa production variables. A- annual yield per tree. B- number of pods produced per tree. C- average pod mass. D- number of beans per pod. Values represent means ± standard error. Different letters above the bars indicate a significant difference.

The annual dry bean yield per tree was significantly reduced by the shelter treatment (Table 2.1, Fig. 2.4. A). Overall, the yield declined from ~1.6 to 1.1 kg tree^{-1} due to sheltering, which, given the density of 1320 trees per ha, would convert into a reduction of roughly 2100 to 1450 kg ha^{-1}. K application had a smaller and positive effect on yield. The number of pods per tree was significantly reduced in the sheltered trees (Table 2.1; Fig. 2.4. A and 2.4. B). Contrary to the sheltering treatment, K application did not significantly affect the number of pods. There were no effects of either sheltering or K treatment on individual pod mass. By contrast, the number of beans per pod was significantly affected by the shelter treatment, K application, and their interaction (Table 2.1). Sheltered trees had fewer beans per pod (Table 2.1 and Fig. 2.4. D). Conversely, K application did not lead to an increase in the number of beans per pod in sheltered trees resulting in greater yield (Fig. 2.4. D). Sheltered trees generally had lower bean yields, due to a lower number of pods and generally fewer beans per pod.

2.5 Discussion

This study investigated the effects of reduced soil water availability by intercepting throughfall, K application and their interaction on cocoa trees. Reduced water availability led to decreased total root length and root mass density, with K mitigating the latter effect. Shelters also reduced Gs, leaf characteristics, and yield, while K application tended to have a positive effect on yield. These findings highlight the negative effects of reduced soil water on cocoa traits and suggest a potential role for K in enhancing yield, although its mitigating effect was not consistently demonstrated.

2.5.1 Rainfall reduction reduced soil water availability

Our results show that under normal circumstances, about 65% of the rainfall reaches the soil directly through throughfall and stemflow, with the remaining 35% intercepted by the canopy. This finding is consistent with results for forest ecosystems (Levia and Frost, 2006; Limousin et al., 2008; Mair and Fares, 2010), but is much higher than the 11% reported by Dawoe et al., (2018), and 24% by Opakunle (1989) for cocoa plantations. These differences may result from variation in daily rainfall patterns, evaporation rates, and canopy characteristics (Imbach et al., 1989; Opakunle, 1989; Crockford & Richardson, 2000; Ufoegbune et al., 2010; Carlyle-Moses & Gash, 2011). For instance, denser canopies or rainfall being distributed over less intense (but more frequent) showers could lead to relatively more rainfall evaporating after being intercepted by the canopy. This highlights the significant impact of canopy interception on soil water availability, and the importance of considering interception losses in cocoa cultivation water management strategies, particularly in regions prone to drought.

Variation in the fraction of rainfall reaching the soil between different sites also has implications for crop growth modelling of yield responses to rainfall and associated decision support for irrigation. Most crop growth models, including the CASE2 crop growth model for cocoa (Zuidema et al., 2003), assume that all rain reaches the soil (with daily rainfall as model input). Our results and hose of others (Augusto & de Miranda, 1994; Niether et al., 2018; Dawoe et al., 2018), challenge this assumption by demonstrating that not all rainfall directly infiltrates the soil due to interception by the canopy. This interception leads to a portion of the rainfall being retained in the canopy or evaporating before it reaches the soil. As a result, the models that assume all rainfall reaches the soil may overestimate the amount of water available for cocoa cultivation. If a significant portion of the rainfall is intercepted and does not contribute to soil moisture, then the actual water needs of cocoa plants may be lower than what these models predict.

Interestingly, the shelter treatment not only reduced soil moisture (Fig. A.2.2) during the treatment period but also appeared to have an enduring effect, with soil moisture levels remaining lower than in the control treatment even when the shelters were no longer in use (Fig. A.2.1. C). This can be explained by the fact that months without shelters tended to have lower rainfall, thus prolonging the process of rewetting of the soil i.e water absorption, infiltration, and percolation, after removal of shelters. This 'carryover' effect however is important as it indicates that short-term changes in rainfall patterns can have prolonged consequences on soil conditions (Niether et al., 2017).

2.5.2 Rainfall reduction affects cocoa tree performance

Cocoa trees have a shallow rooting system (Neither et al., 2019), a characteristic which was confirmed by our results (Fig. A.2.3). Roots were concentrated in the top 40 cm depth of the soil which is consistent with the result found by Schwendenmann et al.

(2010). This trait has been related to the relatively low drought tolerance of cocoa as it limits the tree's ability to access deeper, wetter soil layers during dry periods (Kummerow et al., 1982; Nygren et al., 2013; Moser et al., 2010). While deeper root systems could enhance drought tolerance in cocoa (Moser et al., 2010; Smith & de Smet, 2012), there has so far been little exploration of the genotypic variation in rooting depth in cocoa and the use of grafting on scion with deep-rooting system as in fruit trees.

Sheltering reduced root length and mass density, probably impacting effective soil resource exploration (Pinheiro et al., 2005; Moser et al. 2010; dos Santos et al., 2014, dos Santos et al., 2016). This is in contrast with other crop study revealing that less soil water induced the development of more roots (Ahmad & Li, 2021). To our knowledge, no study in cocoa reported on this difference. Typically, plants respond to low soil water by investing more in root production at the expense of shoot and reproductive growth (Génard et al., 2008). It is possible that the observed decrease in total root length and density in our study resulted from assimilate limitations due to drought-induced reductions in canopy photosynthesis (Génard et al., 2008, Moser et al., 2010; Comas et al., 2013; Maguire & Kobe, 2015), and inhibited photosynthate transportation to roots (Hasibeder et al., 2015).

While reduced soil moisture normally triggers adaptive responses in plants like xylem hydraulic signaling and abscisic acid (ABA) production (Liu et al., 2005; Pirasteh-Anosheh et al., 2016), the sheltering treatment may have disrupted these responses by further decreasing soil moisture levels and altering the plant's environment, making it more difficult for the plant to cope with the stress (Liu et al., 2005).

Our results also showed significant, negative effects of rainfall reduction on Gs, leaf size and leaf greenness, all of which reduce tree photosynthesis. However, Adet et al., (2024) found reduction in Gs and sap flux rate (transpiration) which may have led to reduces photosynthesis efficiency. Thus more CO_2 is taken up at the leaf surface per unit of water transpired leading to a higher photosynthetic water-use efficiency (Balasimha et al., 1991; Mensah et al., 2023). K application positively affected Gs, supporting the notion that it plays an important role in stomatal regulation in cocoa seedlings (Anokye et al., 2021). In a complementary study (Adet et al., 2024), K application was found to increase the stem sap flow indicating higher rates of transpiration which could have been mediated by higher Gs.

We found that the reduction in rainfall decreased cocoa leaf greenness and size, potentially indicating drought-induced inhibition of leaf expansion as also reported by (Fanizza et al., 1991; Rolando et al., 2015; Alban et al., 2016; Lahive et al., 2019). The reduction in leaf greenness suggests lower chlorophyll synthesis or increased chlorophyll degradation, impacting gas exchange, light absorption, and photosynthetic rates (Monteoliva et al., 2021; Mensah et al., 2023). Sheltered trees exhibited lower SLA values indicating thicker and/or denser leaves, possibly adapted to reduced rainfall conditions (Salazar et al., 2018). Furthermore, sheltering resulted in a lower flush intensity, potentially reflecting reduced assimilate availability and active responses to minimize water loss. Taken together, these responses may reduce whole-plant carbon gain (Daymond et al., 2002; Carr & Lockwood, 2011).

2.5.3 Rainfall reduction and cocoa yield

The rainfall reduction treatment negatively impacted several reproductive and yield associated traits including flowering intensity, the number of healthy cherelles, pod number, and bean number per pod, which together resulted in a considerable yield reduction. Similar results have been reported on yield responses to water availability by Abdulai et al. (2018a) in West Africa, Gateau-Rey et al. (2018) in South America, Moser et al. (2010) and Wuriandani et al. (2018) in Asia. In our study, trees produced on average ~1 kg of dry bean when water availability was reduced and ~1.6 kg in control soil water conditions, representing ~31% reduction in yields. Despite the reduction, our yields of 1400 - 3000 kg ha^{-1} surpass the national average of 400-800 kg ha^{-1}. This suggests that the experimental conditions significantly boosted productivity above typical cocoa cultivation levels occurring on farms. Moser et al., (2010) found 10% loss in cocoa yield in response to a rainfall reduction of 53% over 13 months in Sulawesi. This discrepancy could be due to our study being conducted in a much drier area (1200 mm annual rainfall vs 2844 mm in Moser et al. (2010). Results of simulations with a physiological crop growth model for cocoa showed that water limitation leads to 50% loss in simulated cocoa bean yield, predominantly because of variation in dry season duration (less than 50 mm of rain during the two driest months) (Zuidema et al., 2005). Our study in which soil water availability was artificially decreased during the wet season only, shows, conversely, that variation in wet season rainfall may also have a major effect on yield. In line with this, it was also found that the El Niño-Southern Oscillation (ENSO)-related reduction in wet season precipitation caused 62% loss of cocoa production in Central Sulawesi, Indonesia (Keil et al., 2008).

The observed variability in cocoa yield responses to alterations in rainfall patterns, as documented in the literature, may be linked to differences in the proportion of rainfall effectively reaching the soil. We therefore highlight the importance of considering the actual soil water input when interpreting cocoa yield outcomes under different environmental conditions. Yield responses to variation in rainfall may therefore not give an accurate assessment of how cocoa actually responds to variation in water availability. We therefore stress that effects of throughfall and stemflow should be more often evaluated.

Sheltering-induced yield reductions were associated with a tendency towards reduction in flowering intensity, and with the production of fewer cherelles and mature pods, which, in turn, also had fewer beans per pod for unfertilized trees. Reductions in flowering were also reported in field-grown cocoa trees as a result of low soil moisture by Sale (1970), and Schwendenmann et al. (2010). In fact, the peak in flowering in most cocoa growing areas coincides with a peak in rainfall (Young, 1983) with increased flowering intensity during the rainy season and a decline in the dry season (Adjaloo et al., 2012).

Our results show no significant effect of either the shelter or K treatment on cherelle wilt even though it tended to be higher in sheltered trees. However, Adet et al. (2024) documented a contrasting effect, where withholding dry-season irrigation resulted in a reduction of cherelle wilt in mature cocoa trees. The differences could be related to the timing at which difference in water deficit was applied. In the presence of mature or older pods, cocoa trees may prioritize directing their resources towards maintaining the existing pods rather than supporting the growth of new cherelles (Adjaloo et al., 2012; Goudsmit et al., 2023). This preference agrees with the carbon allocation theory proposed by Génard et al. (2008), suggesting that trees allocate resources strategically, potentially resulting in an increased cherelle wilt due to the conservation of energy and resources to sustain mature pods (Lass & Wood, 1985; Valle et al., 1990). When drought reduces the overall number of pods, it may also reduce competition between older and younger pods and thus actually reduce cherelle wilt, as may have happened in Adet et al. (2024). The shelter-induced water limitation in the current study occurred during the wet season, a time when most of the pods were still relatively young with little competition between older and younger pods. Cherelle wilt may have been mediated by an overall lack of assimilates and their partitioning to reproductive organs in sheltered trees (Valle et al., 1990; Adjaloo et al., 2012). As noted above, this reduction in photosynthesis was the likely result of the reduced stomatal closure, leaf greenness, leaf size and flushing intensity that were found (Feller & Vaseva, 2014; Baligar et al., 2017; Mensah et al., 2023). The variation in yield appeared to be related to variation in pod number and number of beans per pod. Nonetheless, this relation is less pronounced in the current study compared to the more significant variation observed in previous studies (Adet et al. in revision), suggesting that the use of pod number as yield proxy may work better under some conditions than others.

2.5.4 Interactive effects of potassium and soil water availability on cocoa performance

K application has been shown to enhance drought stress tolerance of cocoa seedlings (de Almeida & Valle, 2007; Djan et al., 2018; Kaba et., 2022). When soil water availability is low, an adequate supply of K was found to help plants maintaining various physiological processes in these studies. In our study, K application had a positive effect, in both control and sheltered conditions on total root length, Gs, flush intensity, and pod number, and tended to have a positive effect on yield (Fig. 2.2 – Fig. 2.5; Fig. A.2.2; Fig. A.2.3). However, these effects tended to be stronger in the control than in the sheltered trees while some effects, such as the positive effect of K application on root mass density, were only apparent in the control trees. In a previous study, the positive effect of K application on yield was only apparent under high water availability and not under dry conditions (Adet et al., 2024). Also, contrary to our hypothesis, we found no indication of K being able to mitigate the negative effects of the rainfall reduction on Gs in cocoa trees (i.e., in the sense of these negative effects being smaller in K-fertilized trees). This is consistent with Adet et al. (2024), who found that K fertilization did not mitigate the negative effects of withholding dry-season irrigation on leaf functioning. Together these findings indicate that K efficiency relies on adequate soil water availability. However, its specific role as a drought-mitigating strategy in mature cocoa trees merits further investigation.

2.6 Conclusion

The reduction in water availability during the wet season induced by the shelters negatively affected cocoa root growth, leaf physiology, morphology, reproduction and cocoa yield. K fertilization significantly increased yield, but did not fully mitigate the negative drought effects on the overall tree performance. These results emphasize the potential implications of increased variation and possible reduction in wet-season precipitation associated with climate change for cocoa yield. They thus highlight the need for water management strategies to mitigate current and future soil water deficit, and to adopt climate-smart practices to enhance cocoa yield resilience in water deficit conditions.

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Appendix

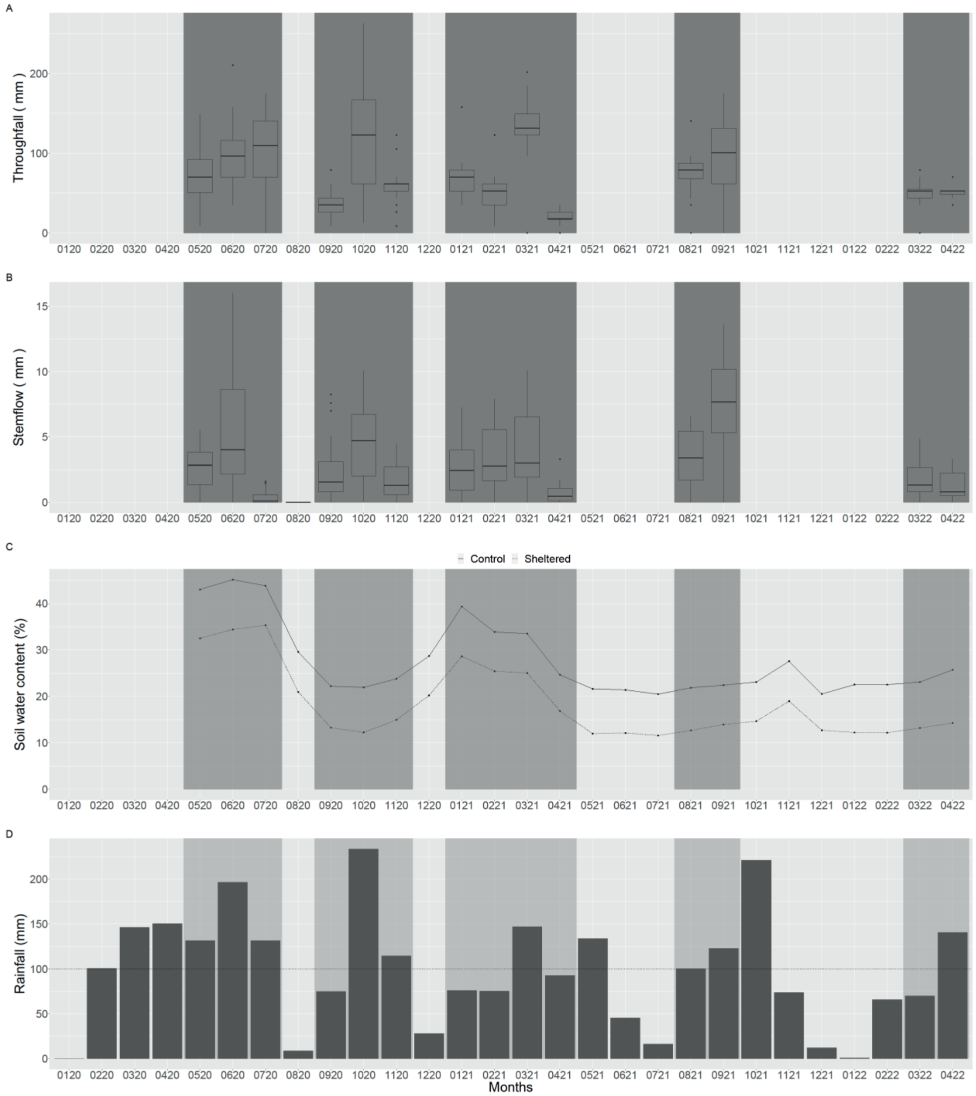


Fig. A.2.1. The monthly rainfall distribution per tree: A- through the canopy, defined as throughfall and B- along the tree stem defined as stemflow, as well as C- the monthly variation in volumetric soil water content, D- the total rainfall received per month in each year. The gray bands indicate the periods in which the shelter was installed in the field. Note that the months during which we recorded stem and throughfall values were the months during which the shelters were in place.

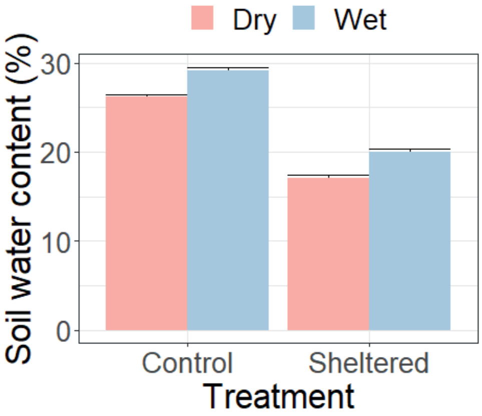


Fig. A.2.2. Effects of the reduced rainfall treatment (Control vs Sheltered) within seasons (dry vs wet) on the soil volumetric water content. Means and standard errors are indicated.

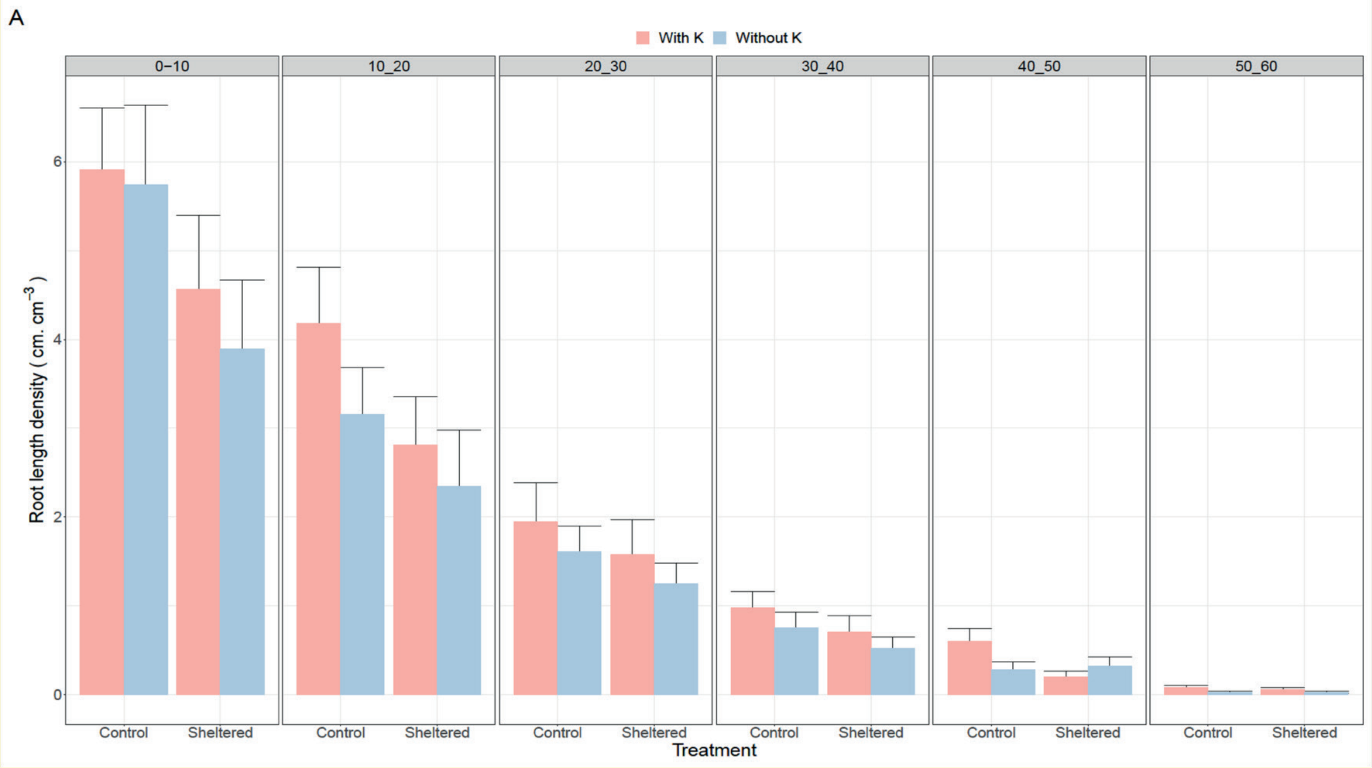
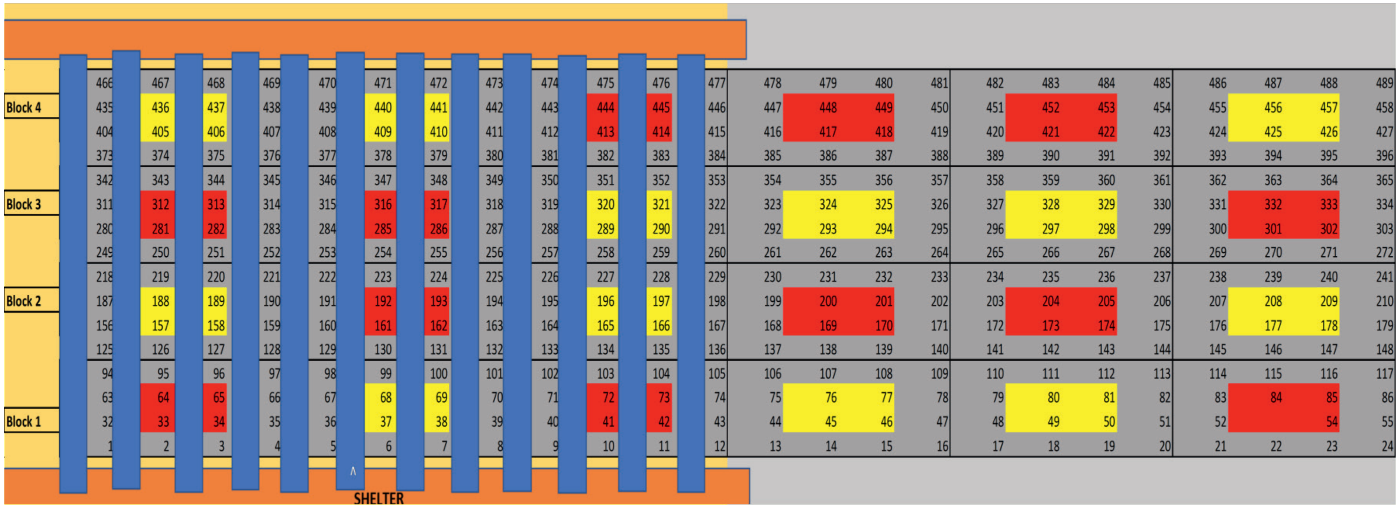


Fig. A.2.3. The effect of the reduced rainfall treatment (Control vs Sheltered) and potassium application (With K vs Without K) on root length density at different soil depths. Means and standard errors are indicated



	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489
Block 4	435	🟨 436	🟨 437	438	439	🟨 440	🟨 441	442	443	🟥 444	🟥 445	446	447	🟥 448	🟥 449	450	451	🟥 452	🟥 453	454	455	🟨 456	🟨 457	458
	404	🟨 405	🟨 406	407	408	🟨 409	🟨 410	411	412	🟥 413	🟥 414	415	416	🟥 417	🟥 418	419	420	🟥 421	🟥 422	423	424	🟨 425	🟨 426	427
	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396
	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365
Block 3	311	🟥 312	🟥 313	314	315	🟥 316	🟥 317	318	319	🟨 320	🟨 321	322	323	🟨 324	🟨 325	326	327	🟨 328	🟨 329	330	331	🟥 332	🟥 333	334
	280	🟥 281	🟥 282	283	284	🟥 285	🟥 286	287	288	🟨 289	🟨 290	291	292	🟨 293	🟨 294	295	296	🟨 297	🟨 298	299	300	🟥 301	🟥 302	303
	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272
	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241
Block 2	187	🟨 188	🟨 189	190	191	🟥 192	🟥 193	194	195	🟨 196	🟨 197	198	199	🟥 200	🟥 201	202	203	🟥 204	🟥 205	206	207	🟨 208	🟨 209	210
	156	🟨 157	🟨 158	159	160	🟥 161	🟥 162	163	164	🟨 165	🟨 166	167	168	🟥 169	🟥 170	171	172	🟥 173	🟥 174	175	176	🟨 177	🟨 178	179
	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148
	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117
	63	🟥 64	🟥 65	66	67	🟨 68	🟨 69	70	71	🟥 72	🟥 73	74	75	🟨 76	🟨 77	78	79	🟨 80	🟨 81	82	83	🟥 84	🟥 85	86
Block 1	32	🟥 33	🟥 34	35	36	🟨 37	🟨 38	39	40	🟥 41	🟥 42	43	44	🟨 45	🟨 46	47	48	🟨 49	🟨 50	51	52	🟥	🟥 54	55
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Fig. A.2.4. Diagram of the experimental design including the experimental units with cocoa trees within the rainfall reduction experiment (blue bands). The red blocks indicate fertilized trees, and the yellow blocks indicate trees without potassium application. Experimental units are separated by lines of border trees.

Table A.2.1. Rainfall partitioning during months when water availability was experimentally reduced by shelters. The table contains monthly (and total) values of rainfall, throughfall, rainfall intercepted by shelters (RIS), stemflow, rainfall lost by canopy interception, rainfall reaching the soil surface in sheltered plots (RRS), and rainfall reaching the soil surface in control plots (RRC). Months not monitored (MN)

Months	Year	Rainfall	Rainfall during monitored months	Throughfall	RIS	Stemflow	Interception Loss in control	Interception Loss in shelter	RRS	RRC	RRS/RRC	RRS	RRC
		mm	mm	mm	mm	mm	mm	mm	mm	mm	%	% of rainfall	% of rainfall
January	2020	0	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
February		100.7	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
March		146.5	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
April		150.5	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
May		131.8	131.8	72.9	48.9	2.6	58.9	107.8	26.6	75.5	35.2	20%	57%
June		196.5	196.5	98.2	65.8	5.7	98.3	164.1	38.1	103.9	36.7	19%	53%
July		131.8	131.8	103.7	69.4	0.3	28.1	97.5	34.6	104	33.3	26%	79%
August		8.7	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
September		74.8	74.8	36.4	24.4	2.2	38.4	62.8	14.2	38.5	36.9	19%	51%
October		233.6	233.6	120.9	81	4.6	112.7	193.7	44.5	125.5	35.5	19%	54%
November		114.5	114.5	57.4	38.4	1.8	57.1	95.5	20.8	59.1	35.2	18%	52%
December		28	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
Average		**109.8**	**147.2**	**81.6**	**54.7**	**2.9**	**65.6**	**120.3**	**29.8**	**84.4**	**35.3**	**20%**	**57%**
Total		**1317.4**	**883**	**489.4**	**327.9**	**17.2**	**393.6**	**721.5**	**178.7**	**506.6**	**35.3**	**20%**	**57%**
January	2021	76.5	76.5	69.8	46.8	3.5	3.2	53.5	26.5	73.3	36.2	35%	96%
February		75.5	75.5	50.8	34	4.3	20.4	58.7	21.1	55.1	38.3	28%	73%
March		147	147	130.5	87.4	3.6	12.9	103.9	46.7	134.1	34.8	32%	91%
April		92.7	92.7	20.8	14	2.6	69.3	85.9	9.4	23.4	40.2	10%	25%
May		134	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
June		45.5	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
July		16.3	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
August		100.3	100.3	76.4	51.2	7.5	16.4	75.1	32.7	83.9	39	33%	84%
September		123.7	123.7	97.2	65.1	1.7	24.8	91.6	33.8	98.9	34.2	27%	80%
October		221.2	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
November		74	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
December		12.4	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
Average		**93.2**	**102.6**	**74.3**	**49.8**	**3.9**	**28.3**	**78.1**	**28.4**	**78.1**	**36.4**	**28%**	**76%**
Total		**1118.2**	**615.7**	**445.5**	**298.5**	**23.2**	**170.2**	**468.7**	**170.2**	**468.8**	**36.3**	**28%**	**76%**
January	2022	0.9	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
February		65.8	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN	MN
March		70.2	70.2	50.8	34	1.27	18.1	53.4	18.07	52.1	34.7	26%	74%
April		140.7	140.7	50	33.5	1.27	89.5	124.2	17.77	51.2	34.7	13%	36%
Average		**69.4**	**105.5**	**50.4**	**33.8**	**1.3**	**55.1**	**88.9**	**17.9**	**51.7**	**34.6**	**17%**	**49%**
Total		**277.6**	**210.9**	**100.8**	**67.5**	**2.5**	**110.1**	**177.6**	**35.8**	**103.3**	**34.7**	**17%**	**49%**

Chapter 3

Genotypic differences in water deficit effects on leaf and crown traits in mature field-grown cocoa

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Abstract

Cocoa trees are highly sensitive to water stress but these negative effects may differ genetically and may be mitigated by potassium (K) application. We studied these effects on six-year-old adult trees, five produced by somatic-embryogenesis, and one by cross pollination (Hybrid), grown under sub-optimal field conditions in central Côte d'Ivoire. Trees were subjected to two soil water treatments (with or without dry season irrigation) and two K fertilization levels (with and without K). We assessed interactive effects of treatments on several leaf and crown traits: sap flux density (SF), leaf water potential (LWP), stomatal conductance (Gs), leaf size (LS), specific leaf area (SLA), leaf water content (LWC), leaf area index (LAI), light interception, litterfall and a visual index of whole-plant water stress (WSI). Mixed-effects model results revealed that withholding irrigation negatively affected SF, LWP, Gs, LS, SLA, LAI and light capture and increased WSI scores. Potassium application did not significantly mitigate the negative effects of withholding irrigation. A significant effect of genotype was observed for most of the monitored leaf physiological, morphological and crown traits. Furthermore, we found significant, positive interactions between genotype and irrigation for SF, LWP, leaf area, LS, SLA, LWC, LAI and intercepted light, suggesting that the differences in observed responses to drought conditions are genotype-specific. These results provide insights into the acclimation strategies of cocoa and genetic variation therein, and can be used to select drought-tolerant genotypes.

Keywords: Cocoa physiology, drought tolerance, genotypic variation, leaf morphology, water deficit

3.1 Introduction

Through increased temperature and shifts in rainfall patterns, climate change is projected to result in increased incidence and severity of extreme climate events such as drought and high temperatures (IPCC, 2018). Many parts of the tropics are hence projected to experience increases in the frequency and the extent of water scarcity events. These changes will likely affect agricultural production in West Africa (Sultan and Gaetani 2016), including production of important commodity crops, such as cocoa (Läderach et al., 2013). In West Africa, where 70% of global cocoa is produced, water availability is the main limiting factor for cocoa growth and yield (Anim-Kwapong and Frimpong 2005; Läderach et al., 2013), resulting in a significant, projected reduction of the area suitable for cocoa production by 2050 (Schroth et al., 2016). Therefore, there is a need to develop climate-smart agricultural adaptation strategies for cocoa production (Lipper et al., 2014 ; Vaast et al., 2016; Nasser et al., 2020).

Cocoa evolved in habitats (Amazon rainforest) that are not typically water limited, probably explaining its drought sensitivity (Wood & Lass, 1987). Cocoa does not tolerate long periods of drought stress (Abdulai et al., 2018a, Bae et al., 2008; Raja & Hardwick, 1988) and possesses large leaves, shallow rooting systems and wide xylem vessels, that allow optimal growth under moist conditions (Antwi, 1994; Meinzer et al., 1992). In cocoa, brief episodes of water shortage can decrease stomatal opening, photosynthesis, and transpiration and may thus reduce yields (Carr & Lockwood, 2011; Gattward et al., 2012; Wessel, 1971). Studies on sap flow density for cocoa showed transpiration was reduced during dry spells (Della Sala et al., 2021; Moser et al., 2010; Abdulai et al., 2018b). Nonetheless, little is known about cocoa transpiration responses to limiting water availability.

The availability of potassium (K) is believed to play an important role in plant responses to drought stress. It regulates cell water potential and affects plant stomatal opening (Jordan et al., 2008; Peiter, 2011), which in turn plays a key role in the way plants deal with water limitation. K application can help to mitigate the negative effect of water deficit on cocoa seedling growth (De Almeida & Valle, 2007; Djan et al., 2017). Hence, K nutrition may also mitigate this effect in mature cocoa, particularly because cocoa has a high demand for K for pod production (Medina & Laliberte, 2017; van Vliet & Giller, 2017). Yet, the interactive effects of water availability and potassium application are poorly understood, and have remained untested in cocoa under field conditions.

Experiments on cocoa drought effects revealed strong genotypic variation to drought tolerance (Daymond and Hadley, 2011), suggesting scope for more drought tolerant cocoa types. However, currently it is not fully assessed how these mechanisms differ across genotypes under field conditions in cocoa plantations and whether some of these genotypes are better adapted to drought stress than others. Therefore, the genetic variation in responses to drought, K and their interaction needs to be further explored, especially in adult trees under field conditions, to support breeding efforts for drought tolerant varieties (Lahive et al., 2018). In this study, we evaluate the influence of water deficit, K application and their interaction on leaf physiology of mature field-grown trees of six cocoa genotypes. We address the following research questions :

(i) How do irrigation and K application and their interaction alter cocoa leaf physiology and morphology?
(ii) How do irrigation and K application and their interaction impact crown-level traits of cocoa?
(iii) Do the effects of irrigation and K application differ among cocoa genotypes?

To this end, we conduct a field experiment in which we test responses to water deficit and K application for adult field-grown trees of six cocoa genotypes: five clones and one hybrid. We hypothesize that: (1) withholding irrigation in the dry season will reduce sap flux density, leaf water potential, stomatal conductance and leaf morphological traits (leaf size, SLA, LWC, leaf area index, intercepted light), and that K application will mitigate these effects for non-irrigated trees; (2) withholding irrigation will result in greater litter production and a reduction in the percentage of intercepted light and leaf area index over the different seasons over the year; and (3) more drought tolerant genotypes will exhibit smaller reductions in sap flux density, water potential and stomatal conductance as well as smaller leaf morphological trait responses and smaller increases in litter production when irrigation is withheld.

3.2 Material and methods

3.2.1 Study site and experimental design

The experiment was conducted at the Nestlé Research station in Zambakro, in the Central-Eastern part of Côte d'Ivoire (6°49′13.98″N, 5°16′36.26″W) from 2020 to 2021. The mean annual temperature at the station was 26.3^{0}C and mean annual precipitation was 1120 mm, which is considered as very dry cocoa producing area (Ehounou et al., 2019). Typically, there are six dry months (precipitation < 100 mm) distributed over a long (November to February) and a short (July to August) dry season. Soil analysis of the experimental site (February 2020 and 2022) showed that the upper soil layer (0 – 20 cm) was acidic, and of sandy loam texture. Soil cation exchange capacity (CEC) ranged between 1-5 (meq 100 g^{-1}) typical for relatively sandy soils. Soil organic carbon tended to be less than 1 mg g^{-1} in the topsoil with 0.83 mg g^{-1} in 2020 and 0.42 mg g^{-1} in 2022.

Planting material was produced through somatic embryogenesis. For each genotype, five subplots of eight trees per row were established in 2015, randomly placed within the overall stand, which contained 240 trees planted at a distance of 2.5 x 3 m. Around the stand, one row of border trees was included.

3.2.2 Experimental treatments

The cocoa stand was divided into two blocks, one irrigated and one not irrigated, and each block was divided into two sub-plots, with one fertilized with potassium and one not. The irrigation and potassium treatments were initiated in January 2020 when the trees were six years old. Water was supplied through drip irrigation. Each tree was located between two emitters (flowrate 1 L h^{-1}) placed on a single drip line. Emitters were located 80 cm away from the trunk and at 80 cm from each other. Irrigation was applied four times per week during dry periods only (Dec-Mar=major dry period and Aug-Sept=minor dry period). It consisted of 9.3 mm day^{-1}, thus a total of 967 mm water supplied per year.

N and P fertilizer was applied either with or without K fertilizer, to increase the probability of K being the main limiting major nutrient. All the genotypes received the same total amount of N and P in the form of Nitrabor (167 kg ha^{-1}) and of Di-

Ammonium Phosphate (DAP, 54 kg ha $^{-1}$). For the potassium treatment, the genotypes received the same amount of K in the form of KCl (71 kg ha^{-1}). Three doses of Nitrabor, DAP and KCl were applied in March, July and September in 2020 and 2021. Each tree received an amount of 0.125 kg N, 0.04 kg P and 0.053 kg K per fertilizer application.

Every month, soil moisture content was recorded from 0 - 20 cm depth at 40 cm diameter from each tree with a time-domain reflectometer (TDR350, FIELDSCOUT Spectrum Technology, Inc.). Daily weather conditions, i.e. rainfall, relative humidity, solar radiation, and air temperature, were monitored at the weather station located near the experimental plot during the experiment from January 2020 to March 2022. For both years, leaf trait and gas exchange measurements (Gs) were done in the middle of both the major and minor dry and wet seasons (December-March, April-July, August-September, October-November) for each genotype over four consecutive days.

3.2.3 Data collection

3.2.3.1 Leaf water potential

Leaf water potential (LWP) at predawn and at midday were measured using a Scholander-type pressure chamber. This was done for four central trees per subplot, per treatment, for two genotypes only, the hybrid Mercedes and the clone CI03 from somatic embryogenesis. Trees were relatively homogeneous, but the two genotypes differed in the way they react phenologically to drought conditions based on field observations. M maintains a green crown, whereas CI03 undergoes an intense crown defoliation. Per tree, six fully developed mature leaves from sun-exposed branches were measured. For measuring midday leaf water potential (MLWP), leaves were excised between 13.00 and 14.00 h, and placed into plastic bags in a cooler, until measurements were done within 10 minutes. For measuring pre-dawn leaf water potential (PLWP), ten sun-exposed mature leaves per species were pre-bagged in the late afternoon of the day before, with both plastic sheet and aluminium foil bags to deflect solar radiation (Choné *et al.*, 2000). Before measurements, the petiole was cut and the bag was closed after removing the air.

3.2.3.2 Sap flux measurements

Thermal dissipation probes (Granier, 1987) were applied to continuously measure sap flux density in cocoa trees every week over four dry months, from February to May 2022. Sap flow was monitored on six cocoa trees in two adjacent genotypes: three central clonal trees (CI03) and three central hybrid trees (M) in each treatment. Each tree was equipped with a pair of needle sensors in holes of 2 mm diameter and about 23 mm depth, below the average jorquette height (~1.3 m) before the first branches. The upper probe of the sensors was heated with a constant power of 12V, and was placed at a vertical distance of 5 cm from the lower, unheated probe. Probes were diagonally installed. Probes were first coated with heat-conducting silicon paste and placed into aluminium tubes pre-inserted in the sapwood. Probes were sealed with reflective bubble wrap and plastic bags to protect the sensors from environmental influences, such as rain and direct solar radiation. Differential voltages of the sensors were measured every 60 s and averaged every 30 min, using an AM16/32 multiplexer and CR1000X data logger (Campbell, Scientific Instruments, Logan, UT). Because of limited capacity of the data logger, measurements could not be done for all trees at the same time. The data logger was transferred from irrigated to non-irrigated plots and vice versa weekly. Sap flow density was calculated following Granier (1987):

$$U = 0.714 \times K \char`^ 1.231 \qquad \text{Eq. (1)}$$

where U is sap flux density (ml cm^{-2} min^{-1}), and K was determined as:

$$K = (\Delta T_M - \Delta T)/ \Delta T \qquad \text{Eq. (2)}$$

where ΔT is the temperature difference between two needles (mV) and ΔT_M is the maximum value of nighttime ΔT (mV) when there is no sap flow (zero set value).

3.2.3.3 Stomatal conductance

Stomatal conductance (Gs) was measured between the veins of the abaxial surface of three sun-exposed, green, healthy, mature leaves per tree for the four central trees per subplot for the M and CI03 genotypes. For both years, Gs was measured in the middle of both major and minor dry and wet seasons (December-March, April-July, August-September, October-November) for each genotype over four consecutive days.

Stomatal conductance was measured with a portable leaf porometer (SC-1 Steady State Leaf Porometer; Decagon Devices, Pullman, WA, United States), which measures the amount of water transpired from the leaves through the stomata. Leaves were allowed to equilibrate, and a reading was recorded after approximately 30–120 seconds. Measurements on leaves that took longer than 3 minutes to equilibrate were discarded. Measurements were performed between 7:00 am and 9:30 am at dew point, before the increase in air temperature.

3.2.3.4 Other leaf traits

Additional leaf traits were measured for each of the six trees for all six genotypes per treatment. Leaf trait measurements were done for the two dry and two wet seasons, in the middle of the season. Four leaves were randomly sampled in four directions within the crown of each tree. Leaf fresh weight and leaf thickness were determined. Leaf area was measured by scanning the leaves with a flatbed scanner, and by analysing the images in Image J software (Perez-Harguindeguy et al., 2016). Leaf dry weight was determined after oven-drying the leaves at 65 °C to constant mass. We calculated leaf water content, which is the amount of water per unit leaf dry mass (LWC; in %), and specific leaf area, the amount of leaf area per unit leaf dry mass (SLA; in $cm^2\ g^{-1}$).

3.2.3.5 Light interception

Light interception was measured monthly using a HOBO light sensor (HOBO, USA) on sunny days from 10:30 am to 14:30 pm every month. Firstly, the incident light intensity (I) was measured below the crown (I_{below}) at two positions within each subplot with the instrument (mEssfix 6m, Switzerland) surface horizontal upward, facing the sky. Secondly, incident light intensity (I) was measured above the crown (I_{above}) at the same location. Percent light interception was calculated as follows: % Interception = $[100 - (I_{below} \times 100 / I_{above})]$ Eq. (3), where I_{below} = global incident radiation below the crown, and I_{above} = global incident radiation above the crown. Measuring light availability simultaneously above and below the crown allowed the leaf area index to be calculated by inverting the Beer-Lambert radiation extinction law (Monsi and Saeki, 1953) as follows: $LAI=-1/k \ln (I_{below}/ I_{above})$ Eq. (4). The light extinction coefficient (k) was taken as 0.6 (Zuidema et al., 2005).

3.2.3.6 Litterfall sampling

Litterfall was estimated by randomly placing two 1 m × 1 m litter traps with a 2-mm nylon mesh in each subplot. Litter traps were placed 1 m above the ground. Litter was collected from the 60 traps at monthly intervals, but at weekly intervals in periods of heavy rainfall and/or wind. The collected litter samples were separated into leaf and non-leaf components, and oven-dried at 65 °C for two days to constant mass, and weighed. We counted the number of leaves in each sample.

3.2.3.7 Visual water stress index

During dry periods, cocoa leaves typically turned yellow, dried out and were dropped, to reduce water loss. We used a visual index of water stress based on the degree and severity of leaf discoloration and crown defoliation. A score of 0 – 5 was assigned to indicate water stress levels (0 not stressed and 5 most stressed): 5 indicated fully defoliated trees, 4 indicated 80-100% of the leaves dried, 3 indicated 75-80% of the leaves dried, 2 indicated 50-75% of the leaves dried, 1 indicated 25-50% of the leaves dried, and 0 indicated <25% of the leaves dried. In addition, we monitored crown phenology over the months. For each tree, the presence or absence of each of the following stages was recorded in the first week of each month: leaf flushing, mature, dark green leaves and senesced leaves (yellow or turning yellow), flowers, and fruits.

3.3 Statistical analysis

In the analysis, the dry period was defined as the period in which irrigation was turned on, and the wet period was the period in which irrigation was turned off. We used linear mixed-effects models (LMMs) in order to assess the effect of irrigation, K application and their interaction on SF, G_s, PLWP, MLWP, the water stress index, litterfall rates and leaf and crown traits, and how effects differed between genotypes. In a first step, we tested whether there was an effect of the period (dry vs. wet), irrigation and their interaction, based on a mixed-effects model comparison. We included the period (dry/wet), irrigation and the two-way interaction between period and irrigation as fixed effects, with tree, and litter trap in the case of litterfall, as random effect. We compared models with all possible combinations of the fixed effects using maximum likelihood estimation, and selected the best model based on Akaike's Information Criterion (AIC_c), adjusted for small sample sizes. The model with the lowest AIC_c value was selected. Model assumptions of the LMMs were checked by inspecting residual plots for homogeneity and quantile-quantile plots for normality. Marginal and conditional R squared values were calculated for the best model (Nakagawa and Schielzeth 2013), where the marginal R squared indicates variation explained by the fixed effects only, and the conditional R squared indicates variation explained by both the fixed and the random effects.

In a second step, we included the dry period only to evaluate the effects of irrigation, potassium application and genotype, using linear mixed-effects models. Genotype, potassium application, irrigation treatment and the interactions between genotype and potassium, genotype and irrigation, irrigation and potassium, and genotype and irrigation and potassium were included as fixed effects. Tree, and litter trap in the case of litterfall, were included as random effects. Similar to the first analysis, a model comparison was conducted, and we selected the best model based on AIC_c. Effects were considered significant, if bootstrapped 95 % confidence intervals of the model coefficients did not overlap with zero. PostHoc tests were only performed in case of a significant interaction. All statistical analyses were performed using R Statistical Software 3.5.1 (R Core Team, 2018). LMMs were performed using the "glmmTMB" package (Brooks et al., 2017).

3.4 Results

3.4.1 Microclimate variation

Rainfall generally showed a bimodal pattern typical for this region, with high values during March - June and August - September, and low values during November - February (Fig. 3.1. A). During January 2021, however, exceptionally high rainfall was reported (496 mm). The mean relative humidity was lowest in the dry periods in 2019 (on average 81.7±4.5 %), in 2020 (on average 81.3±5.5 %), and in 2021 (on average 76±3.8 %) and highest in wet periods (Fig. 3.1. A). The monthly relative amount of time under sunny conditions ranged from 20-30% to 85% and tended to be higher in the period between February and August than in the other months (Fig. 3.1. B). The average diurnal air temperature followed a pattern that was similar to that of the solar radiation and ranged between 26.5 ± 1 ºC (mean ± SE) to 30 ± 2 ºC (Fig. 3.1. B). Soil moisture content (VWC) was highest in March and November 2020, and in April and December 2021 VWC ~35% (Fig. 3.1. C). VWC was higher in wet periods and in irrigated plots and lower in the non-irrigated plots (Fig. 3.1. C).

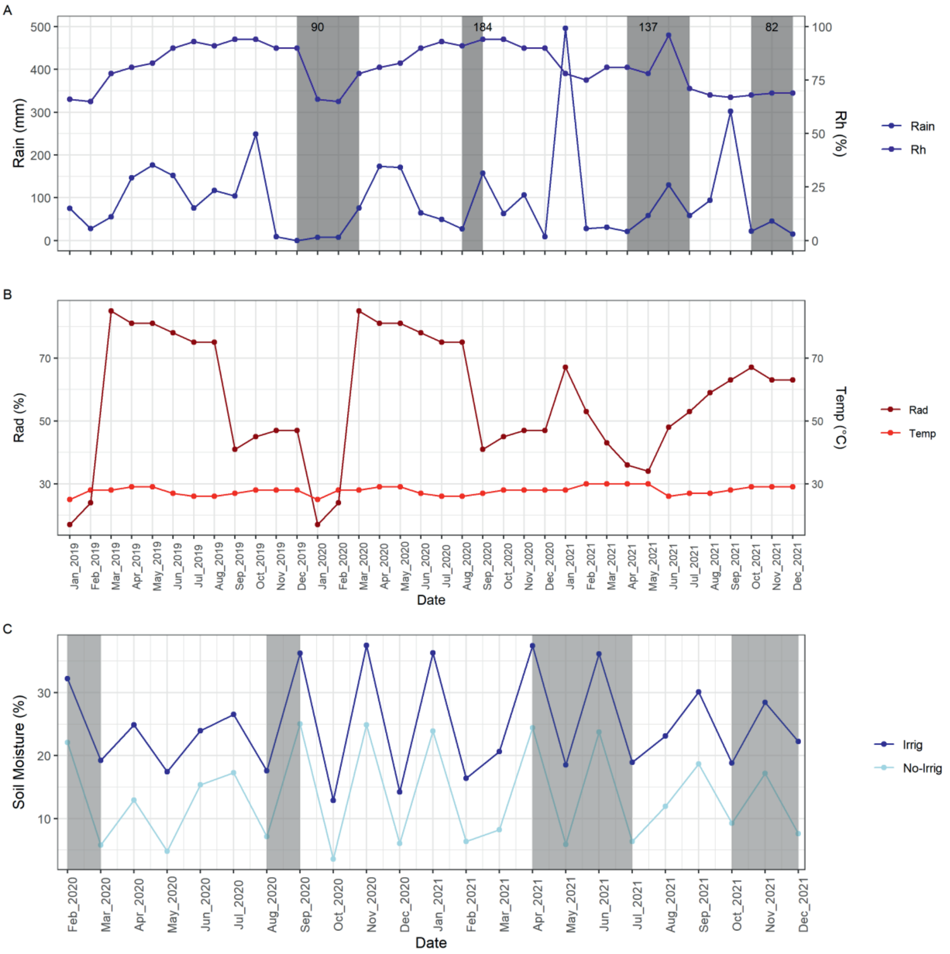

Fig. 3.1. Monthly averages of microclimate variables throughout the experiment. (A) Rainfall (Rain; mm) on the left y-axis and relative humidity (Rh; %) on the right, (B) Solar radiation (the percentage of time with sunshine Rad; %) on the left y axis and air temperature (Temp; °C) on the right axis, (C) Soil volumetric water content (%) for

irrigated (Irrig) and non-irrigated (No-Irrig) plots. The grey areas indicate the dry months in which the irrigation treatment was applied; the numbers in the grey area indicate the total rainfall received in that period.

3.4.2 Effects of irrigation in the dry and the wet period

We first tested whether there was an effect of the period (dry vs. wet), irrigation and their interaction on different traits combining the data for different genotypes and potassium treatments. Leaf water potential values were significantly lower (more negative) in the dry than in the wet period, and irrigation significantly increased leaf water potential values in the dry season. This effect extended into the wet period even though there was no irrigation supply in the wet season (Fig. A.3.1). Stomatal conductance was significantly lower in the dry than in the wet period, and higher under irrigation than without. The negative effect of dry periods on Gs was mitigated by irrigation (as indicated by the significant period by irrigation interaction) (Fig. A.3.1). Leaf area was significantly higher in the wet than in the dry period and higher in irrigated than non-irrigated trees (Fig. A.3.2). Notably, there was a significant interaction between irrigation and period, with a slightly larger irrigation effect in the wet than in the dry period. Specific leaf area (SLA) and leaf water content (LWC) were both significantly influenced by the effect of irrigation and the effect of period separately, while leaf thickness was only influenced by period, being larger in the wet than in the dry period (Table A.3). Most of the crown traits responded to irrigation with irrigated plants having higher LAI, light interception and lower WSI (less stressed) than non-irrigated ones. LAI, light interception and WSI were lower in the dry than in the wet period (Table A.3). Litterfall was larger in the dry period than in the wet period for non-irrigated trees, as indicated by the significant interaction between period and irrigation (Fig. A.3.3). Hereafter, we include analyses for the dry period only.

3.4.3 Effects of irrigation, potassium application and genotypes on leaf physiological traits

We assessed how leaf water potential and stomatal conductance in cocoa trees responded to the irrigation and potassium treatments during the dry period for genotypes M and CI03 only. Results revealed a significant effect of genotype, irrigation as well as a significant interaction between genotype and irrigation on leaf water potential at predawn (pLWP) and midday (mLWP). The irrigation treatment made the leaf water potential significantly less negative. However, no significant effect of potassium and no interaction between the potassium and irrigation treatments was found (Table 3.1, Fig. 3.2. A, B). The water potentials of the two genotypes responded differently to irrigation. With mean values of -15.11 to -16.8 Bar, the genotype M had a higher (less negative) average leaf water potential (pLWP and mLWP) values compared to CI03 (-16.3 to -18.06 Bar), and this difference was larger in the irrigated than in the non-irrigated trees (Fig. 3.2. A, B, Table A.3). This indicates that genotype M exhibited both overall less negative LWPs, and a greater change in LWP in response to variation in water supply, with a probably faster recovery from water stress than CI03.

As expected, irrigation significantly increased stomatal conductance (Gs), and it did so in both genotypes (Fig. 3.2. C). Potassium application did not affect stomatal conductance, and we did not find any significant interaction between potassium and irrigation. Stomatal conductance significantly differed among the two genotypes, being smaller for CI03 (114.18 mmol s^{-1}) than for M (146.15 mmol s^{-1}). Responses to irrigation were similar for both genotypes, as the interaction between genotype and irrigation was not significant.

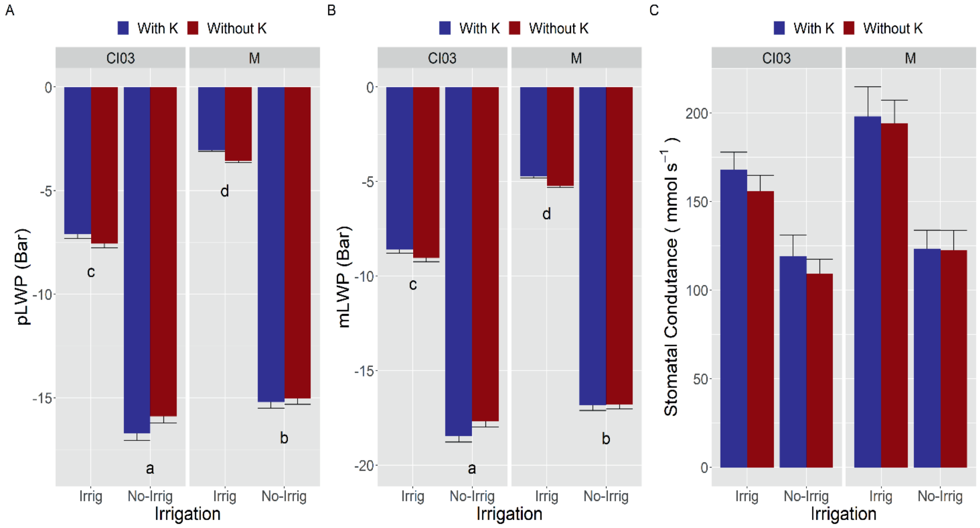

Fig. 3.2. Effects of irrigation, potassium fertilization, and genotype on leaf water potential and stomatal conductance for mature trees of two cocoa genotypes. Genotypes refer to one clonal variety CI03 and one hybrid variety M. (A) Predawn leaf water potential (pLWP, Bar), (B) Midday leaf water potential (mLWP, Bar), (C) Stomatal conductance (mmol/s). "Without K" indicates without potassium application, "with K" indicates with potassium application, "Irrig" indicates the irrigated treatment and "No-Irrig" indicates the non-irrigated treatment. Values represent means ± standard error. Letters above the error bars indicate the Compact Letter Display (cld) of the statistically significant different groups after a Tukey HSD Post-hoc test for multiple pairwise comparisons of the groups.

Table 3.1. Linear mixed-effects model results testing the effects of genotype, irrigation, potassium fertilization and their interactions on cocoa tree physiology responses. Included variables were predawn leaf water potential (pLWP), midday leaf water potential (mLWP), stomatal conductance (Gs), sap flux density (SF), leaf area, specific leaf area (SLA), leaf thickness, leaf water content (LWC), leaf area index (LAI), intercepted light percentage, water stress index (WSI), and litterfall. Fixed effects that were tested in the model comparison are indicated: genotype (Gen), potassium treatment (PtT), irrigation treatment (Irrig), the interaction between predictors (:), the marginal (m) and conditional (c) R squared values. + indicates that the predictor was included in the best model. $^{\text{Sig}}$ indicates that the effect of the predictor was significant.

Variables		**Unit**	**Gen**	**PtT**	**Irrig**	**Gen: PtT**	**Gen: Irrig**	**PtT: Irrig**	**Gen: PtT: Irrig**	**R^2m**	**R^2c**
Leaf physiological traits	pLWP	Bar	+ $^{\text{Sig}}$	+	+ $^{\text{Sig}}$		+ $^{\text{Sig}}$	+		0.37	0.37
	mLWP	Bar	+ $^{\text{Sig}}$	+	+ $^{\text{Sig}}$		+ $^{\text{Sig}}$	+		0.57	0.60
	Gs	mmol s^{-1}	+ $^{\text{Sig}}$	+	+ $^{\text{Sig}}$		+			0.02	0.02
Sap flux density	SF	ml cm^{-2} min^{-1}	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+	+			0.21	0.09
Leaf morphological traits	Leaf area	cm^2	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$		+ $^{\text{Sig}}$	+		0.13	0.21
	SLA	cm^2 g^{-1}	+ $^{\text{Sig}}$		+	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+		0.04	0.15
	Thickness	mm	+	+	+	+	+			0.11	0.17
	LWC	%	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$		0.04	0.15
Crown traits	LAI	_	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$			0.08	0.32
	Intercepted	%	+ $^{\text{Sig}}$	+	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$	+ $^{\text{Sig}}$			0.23	0.23

Light					
WSI	_	+ ^{Sig}	+ ^{Sig}	0.11	0.11
Litterfall	kg ha^{-1}	+ ^{Sig}	+	0.11	0.54

3.4.4 Sap flux density

We assessed whether sap flux density in cocoa trees responded to the potassium and irrigation treatments during the dry period in the genotypes M and CI03, as sap flux measurements were done in the dry period only. We found a significant effect of genotype, irrigation, and potassium application on the mean water flux density. The irrigation treatment significantly increased mean water flux density (Table 3.1, Fig. 3.3.), but there was no significant interaction between irrigation and potassium. The mean water sap flux density was significantly reduced from 0.450 to 0.416 ml cm^{-2} min^{-1} under non-irrigated conditions. Genotype M had higher average flux density values than CI03 (Fig. 3.3.). There was no significant genotype by irrigation interaction. Potassium increased mean water flux density by about 11 %, 14.28% in M and 22.22% in CI03, and this response did not differ significantly between genotypes.

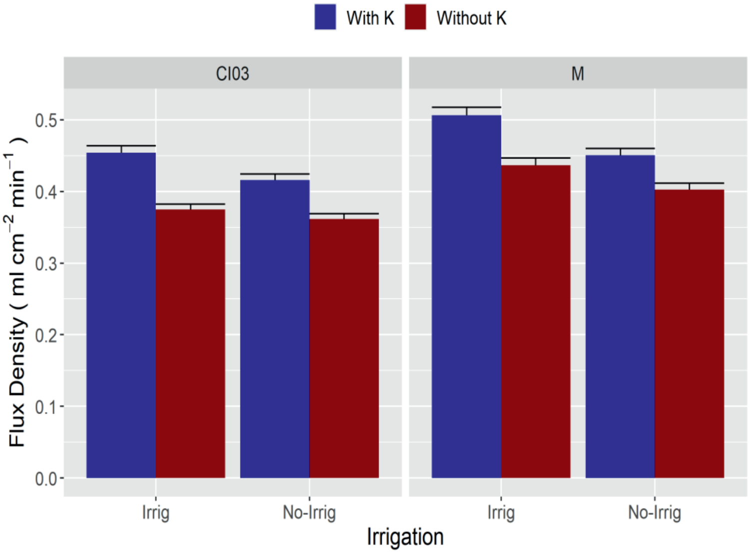

Fig. 3.3. Effects of irrigation, potassium fertilization, and genotype on sap flux density (SF) for two cocoa genotypes. Genotypes refer to one clonal variety CI03 and one hybrid variety M. Without K indicates without potassium application, with K indicates with potassium application, Irrig indicates with irrigation and No-Irrig indicates that no irrigation was applied. Values represent means ± S.E.

3.4.5 Leaf morphological traits

We assessed the effect of irrigation and potassium application on leaf morphological traits for all six cocoa genotypes during dry periods. Generally, leaf area (the mean area of a leaf) significantly increased in response to irrigation, but the response differed among genotypes (Fig. 3.4. A), as there was a significant interaction between genotype and the irrigation treatment. There was a significant effect of genotype on specific leaf area (SLA) with a significant interaction between genotype and potassium application, and between genotype and irrigation (Table 3.1). Overall, there was no clear response to irrigation and potassium application in SLA values (Fig. 3.4. B), as responses strongly differed among genotypes. Irrigation and potassium application did not have a significant effect on leaf thickness (Table 3.1, Fig. 3.4. C), and there was no difference between genotypes. We did find a significant effect of genotype, irrigation, and potassium application on leaf water content (LWC), as well as interactions between genotype and potassium, between genotype and irrigation, and between potassium and irrigation (Table 3.1). Generally, responses were rather weak and differed across genotypes (Fig. 3.4. A, Fig. 3.4. D).

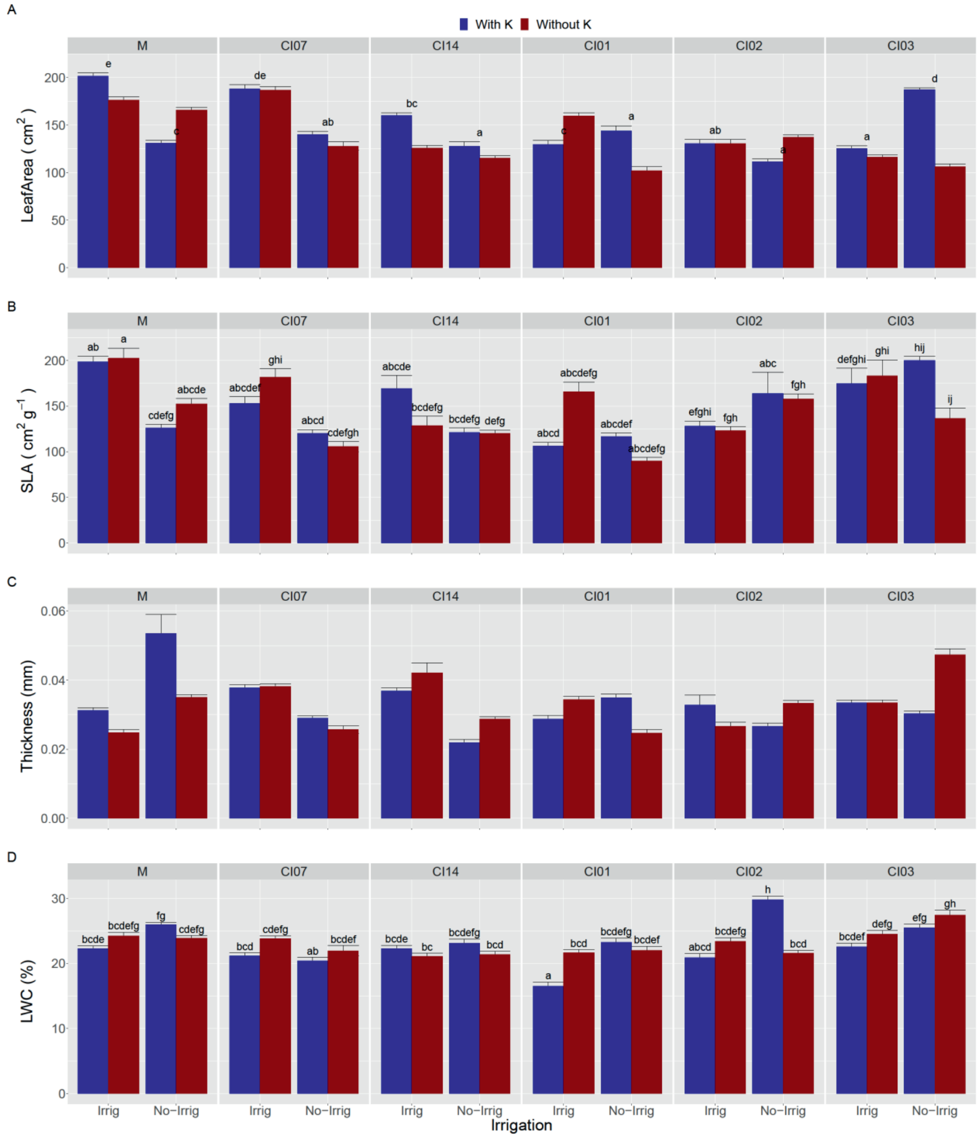

Fig. 3.4. Effects of irrigation, potassium application and genotype on leaf morphological traits: (A) Leaf area (cm^2), (B) Specific leaf area (SLA, cm^{-2} g^{-1}), (C) Leaf thickness (mm), (D) and leaf water content (LWC, %) for six genotypes. Genotypes refer to five clonal varieties CI07, CI14, CI01, CI02, CI03 and one hybrid variety M. Treatment abbreviations as in Figure 3.2. Letters above the error bars indicate the Compact Letter Display (cld) of the statistically significant different groups after a Tukey HSD Post-hoc test for multiple pairwise comparisons of the groups.

3.4.6 Crown dynamics

We evaluated performance of cocoa genotypes in response to irrigation and potassium application at the crown level. Leaf area index (LAI) was significantly influenced by genotype, potassium and irrigation, with significant two-way interactions between genotype and potassium application, and between genotype and irrigation (Table 3.1). Generally, LAI declined significantly when irrigation was withheld as shown by a significant main effect (Table 3.1). But when testing per genotype, the irrigation effect was mostly not significant (Fig. 3.5. A). The percentage of intercepted light was significantly affected by genotype, irrigation, the interactions between genotype and potassium application, and between genotype and irrigation. As with LAI, the no-irrigated trees had lower light interception values than the irrigated trees, but this effect was weak, and was not present for most of the genotypes (Fig. 3.5. B). The effects of potassium on LAI and light interception were fairly inconsistent being positive in some but negative in other genotypes (Figs 3.5. A, B).

WSI was significantly increased by withholding irrigation, indicating more drought stressed plants, and differed among genotypes (Table 3.1). This increase in WSI tended to be larger in the CI02 and CI03 clones than in the others, but the genotype by irrigation interaction was not significant (Fig. 3.5. C). There was neither a significant effect of potassium nor a significant interaction between irrigation and potassium application on WSI (Table 3.1).

Defoliation occurred during dry periods, and lasted between 4 and 11 weeks (depending on the year) in all genotypes, leading to increased litterfall. Subsequently, re-growth of leaves occurred immediately at the onset of wet periods (data not shown). Litterfall significantly differed among genotypes, but we did not find significant effects of irrigation, potassium application, or their interaction (Table 3.1). M and CI01 produced significantly less litterfall than CI07, CI03, CI14 and CI02 (Fig. 3.5. D).

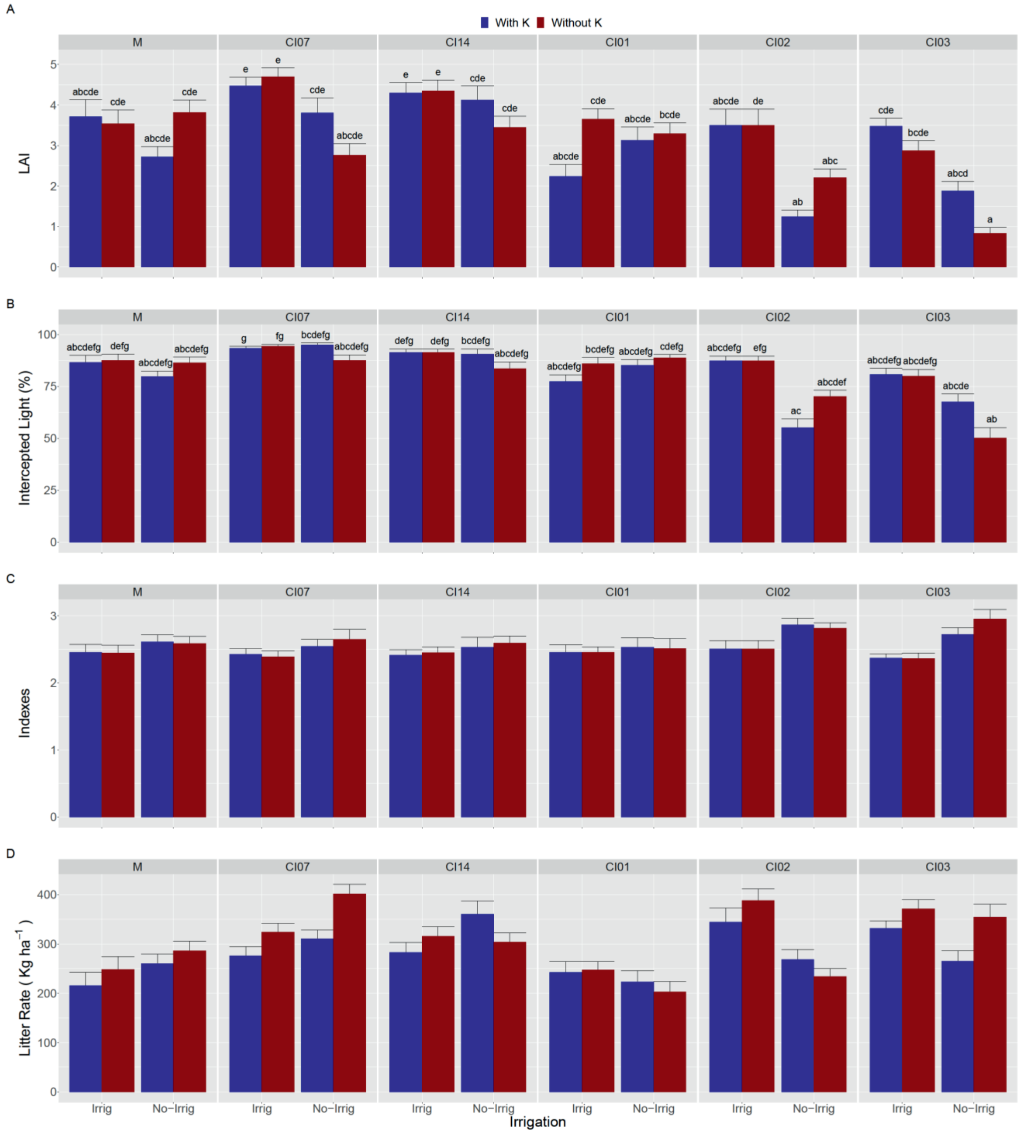

Fig. 3.5. Effects of irrigation and potassium application on the performance of cocoa genotypes on (A) leaf area index (LAI), (B) Percentage of light intercepted (%) by the crown, (C) water stress index (WSI) and (D) monthly litterfall. Treatment abbreviations as in Figure 3.2.

3.5 Discussion

Physiological responses of cocoa genotypes to variation in irrigation and potassium application were evaluated under field conditions to test how leaf and crown characteristics of mature field-grown trees respond to water deficit, and whether these responses were modified by potassium application. We also explored whether these responses differed across genotypes.

Overall, we found that withholding irrigation had clear negative effects on cocoa sap flow density (SF), several leaf physiological water-related traits (pLWP, mLWP, Gs), on leaf area, as well as on crown size (LAI). Furthermore, withholding irrigation increased the overall tree water stress index (WSI). The strongest LAI reductions in the non-irrigated treatment were observed for genotypes CI02 and CI03, suggesting these to be less drought tolerant than the other genotypes. We also found that the hybrid M was able to maintain higher levels of LWP both under irrigated and non-irrigated conditions than the apparently less drought tolerant CI03 clone. This was not the case for sap flux and Gs, which did not show interactions between genotype and irrigation.

Together these results indicate a clear genetic variation in drought tolerance that is reflected at both leaf and crown levels. However, contrary to our expectations, there were no consistent interactive effects between irrigation and potassium application for any of the included traits at the leaf, crown and whole-tree level. We did find that potassium application increased sap flux density, which may indicate a role for potassium in relieving drought stress.

3.5.1 How cocoa trees deal with water deficit at leaf level

As hypothesized, withholding irrigation had negative effects on leaf physiological and leaf morphological traits. Our findings showed a significant reduction of withholding irrigation on daily sap flux density (SF) and on predawn and midday leaf water potential (Table 3.1). Similar findings were obtained for clonal cocoa seedlings (Santos et al., 2018; Osorio-Zambrano et al., 2021) at a soil volumetric water content (VWC) below 6%. In another study, Tezara et al., (2020) noted that drought caused a 40% decrease in LWP of cocoa seedlings. Because LWP predominantly reflects the ability of plants to acquire water, lower values are an indication of drought stress (Lambers et al. 1998) and inhibit leaf physiological functioning. To put our results in context, the LWP values under non-irrigated conditions in the dry season were about -16 bar, which for cocoa is considered the threshold for cessation of leaf photosynthesis (De Almeida & Valle, 2007). In addition to indicating drought stress, the reduction in LWP may also (but to a much lesser extent) be due to accumulation in leaf cells of osmolytes resulting in a more negative osmotic potential. As water moves into the plant along a pressure gradient, this osmotic response facilitates water uptake under drought conditions. However, we did not measure leaf osmotic potential and hence cannot assess the role that variation herein might have played.

Results show that Gs was significantly reduced under withholding irrigation in both genotypes M and CI03 (Fig. 3.2. C), which was also found in studies on other cocoa genotypes (Acheampong et al, 2013; Araque et al., 2012; Baligar et al., 2008; Daymond et al., 2011; De Almeida et al., 2016; Tezara et al., 2016). Stomatal closure helps reducing water loss and preventing leaf dehydration. In addition, as transpiration (E) declines more steeply with a reduction in Gs than photosynthesis (A), it may increase photosynthetic water-use efficiency (A/E) (Lahive et al., 2019; Tezara et al., 2020). On the other hand, it also entails a reduction in photosynthesis and transpiration, possibly resulting in leaf heating.

LWC was only slightly lower in the dry than in the wet periods (Fig. A.3) and, surprisingly, there was no consistent effect of irrigation (Fig. 3.3. D). LWC reflects the balance between water availability (indicated by LWP), water transport to the leaves (indicated by SF) and loss through transpiration (indicated by Gs and SF). Inconsistent effects of withholding irrigation on LWC likely indicated that water loss prevention through stomatal closure matched the reduction in water availability such that leaves were not significantly dehydrated (Da Matta, 2004).

Our results did show reductions in SLA and leaf size in dry compared to wet periods (Fig. A.3). Furthermore, responses in these traits to irrigation were inconsistent among genotypes, perhaps as a result of the way we conducted our experiment. We only withheld irrigation during the dry season when trees do not produce many leaves unless soil water content increases (Miyaji et al., 1997a, Miyaji et al., 1997b). As leaf size and SLA are largely determined at leaf initiation and formation, our trait values measured in the dry season may reflect the conditions in the wet season when the leaves were formed. In this respect, our experimental set-up differed from studies which did find a negative effect on these traits, as those studies applied constant drought and leaf formation was thus certainly under drier conditions than in their wet treatments (Ayegboyin & Akinrinde, 2016; Baligar et al., 2017).

3.5.2 Crown-level responses to drought

We expected that drought would result in greater litterfall, WSI, and lower LAI and hence light capture in cocoa trees. Our results confirm this hypothesis for WSI and LAI, but not for light capture and litterfall, which did not clearly differ between irrigation treatments. The latter could be associated with the fact that litter production is a function of both the fraction of leaves that are dropped and the size of the crown (i.e. LAI). Drought resulted in a considerably (~40%) lower LAI, which may result in lower transpiration and maintenance respiration (Gupta et al., 2020; King, 1990; Santos et al., 2014), and increased the water stress index. Results revealed that the reduction in LAI together with the reduction in Gs was largely responsible for the lower SF values observed in non-irrigated plants, which was also found in other studies (Santos et al., 2012 ; Villalobos et al., 2000). Lower LAI and light capture while preventing dehydration during drought events may limit the ability of plants to quickly recover once drought conditions end.

3.5.3 Potassium fertilizer did not mitigate water stress

Contrary to our expectations, application of potassium fertilizer did not generally mitigate the negative effects of withholding irrigation on most of the leaf and crown traits or on the overall stress index. This also contrasts with other studies where a drought mitigation effect of potassium fertilizer application was observed in cocoa, albeit in seedlings (Anokye et al., 2021; De Almeida & Valle, 2007; Djan et al., 2017; Kaba et al., 2022). The only significant potassium effect we found was on daily sap flux density (SF), which increased with potassium application. In a study on *Eucalyptis* *grandis* trees, sap flow was positively related to whole-plant transpiration and hence to LAI and Gs (Asensio et al., 2020). In our study, LAI did increase with potassium application in the clone CI03, but not in the hybrid M, while GS was not affected by potassium in either genotype. Regarding the latter it is worth noting that we only measured Gs in the morning while SF reflects daily transpiration, it could thus be that potassium application may have reduced midday stomatal closure (Oddo et al., 2020).

As noted by Lahive et al. (2019), in cocoa the interactive responses to water and potassium availability could be genotype-specific, i.e., being more apparent in some genotypes than in others. It could thus be that the presently selected genotypes happened to be ones that do not show this response very strongly. Another explanation for our findings could be a reduction in root hydraulic conductance that may have reduced potassium ion transport from the soil into the plant (Qi et al., 2019; Steudle, 2000). Finally, and possibly more importantly, our experiment was initiated five months after cessation of fertilizer application to all plots on this site. It is possible that trees may still have had stored potassium reserves and that use of these reserves masked any potassium fertilizer effect in our study. Yet, this does not explain the higher sap flux rates in the potassium treatment. Unfortunately, we were not able to obtain a sufficient amount of samples to test for effects of the treatment on potassium concentrations in soil and leaves. In line with (Lahive et al., 2019), we stress that more and longer-term research is needed to explore the potential of potassium application as a drought stress mitigating strategy in adult field-grown cocoa.

3.5.4 Genotypic differences in responses to drought

We observed clear genotypic differences in the effects of withholding irrigation on LAI, namely that the increment in these values was significantly lower in the genotypes CI02 and CI03 than in the other genotypes. The whole-plant stress indicator WSI tended also to be significantly larger for these two genotypes. This suggests that CI02 and CI03 were less drought tolerant than the other four genotypes. This result is consistent with that of other studies documenting cocoa genetic variation in drought tolerance (Araque et al., 2012; Ávila-Lovera et al., 2016; Daymond et al., 2011).

Genotype by irrigation interactive effects were also observed on leaf physiological and morphological traits. The apparently more drought tolerant hybrid M maintained less negative LWP values under non-irrigated conditions and exhibited a larger LWP difference between irrigated and non-irrigated conditions than the apparently less tolerant CI03, with similar trends being observed for Gs. A relatively high (less negative) midday LWP during drought has been proposed as a proxy for drought tolerance (Araque et al. 2012). The ability to maintain less negative LWP values under drought conditions can be associated with the presence of deep, dense root systems; and/or resistance to losses in stem water conductance (De Almeida & Valle, 2007; dos Santos et al., 2016).

Together, the results for SF, LWP and GS indicate that the genotype M tends to maintain better access to water when irrigation is withheld and shows a stronger positive response in these traits when irrigated. The latter suggests a faster recovery and hence greater resilience under drought stress of hybrid M than clonal variety CI03. Generally, SF was lower in CI03 than in M (Fig. 4.). This lower SF under water limitation aligns well with a previous study (Abdulai et al., 2018b) that demonstrates a reduction in daily maximum cocoa sap flux density in full sun conditions at the end of the dry period. The studies by Dierick et al. (2010) and Dierick & Hölscher (2009) demonstrated that daily cocoa sap flux density declined in response to high levels of vapor pressure deficit (VPD), and that soil water content decreased strongly differ among species monitored (Köhler et al., 2009). Differences among genotypes could be driven by differences in stem xylem vessel features such as vessel area, density, and diameter that could be smaller in CI03 than in M. In apple, for example, lower SF was associated with smaller xylem vessel features (Bhusal et al., 2019). Overall, our results suggest that cocoa genotypic differences in drought tolerance reflect plasticity differences in traits determining drought responses.

3.6 Conclusion

Physiological responses to water deficit and potassium application were evaluated in field conditions for six cocoa genotypes derived from somatic embryogenesis. Withholding irrigation significantly affected cocoa sap flux density, leaf physiology, leaf morphology and crown traits, while potassium application did not mitigate the negative effects of water deficit. Therefore, the putative, added benefit of potassium application to mitigating drought stress needs to be further investigated, in long-term fertilizer trials. Cocoa responses to irrigation were strongly genotype-specific, Mercedes (M) followed by the clone CI07 seem to have greater adaptive ability to water deficit than the other genotypes. Results imply that the observed genotypic variation in responses to irrigation provides useful input for developing cocoa varieties specifically suitable for restricted (limited to the dry period) irrigation practices. Together these results contribute to the body of knowledge on how strategic combinations of dry-period irrigation and use of drought tolerant genotypes could help in making cocoa production more drought resilient.

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Appendix

Table A.3 Linear mixed-effects models (lowest AICc) testing the effects of seasons, withholding irrigation, and their interactions on mature cocoa trees physiological responses, the marginal (m) and conditional (c) R squared values. + indicates that the predictor was included in the best model. ^{Sig} indicates that the effect of the predictor was significant.

Variables		Unit	Period	Irr	Period: Irr	R^{2}m	R^{2}c
Leaf physiology traits	pLWP	Bar	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	0.53	0.57
	mLWP	Bar	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	0.56	0.59
	Gs	mmol s^{-1}	+ ^{Sig}		+^{Sig}	0.06	0.06
Leaf morphology traits	Leaf Area	cm^2	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	0.21	0.44
	SLA	cm^{2}g^{-1}	+ ^{Sig}	+ ^{Sig}		0.02	0.17
	Thickness	mm	+ ^{Sig}			0.04	0.33
	LWC	%	+ ^{Sig}	+ ^{Sig}		0.03	0.43
Sap flux density	SF	ml cm^{-2} min^{-1}	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	0.003	0.008
Crown traits	LAI	_		+ ^{Sig}		0.24	0.32
	Intercepted Light	%	+	+ ^{Sig}		0.06	0.22
	WSI	_	+	+ ^{Sig}		0.11	0.11
	Litterfall	kg ha^{-1}	+ ^{Sig}	+	+ ^{Sig}	0.11	0.54

*^{Sig} means significant effects of the predictors associated with each modelling inclusion

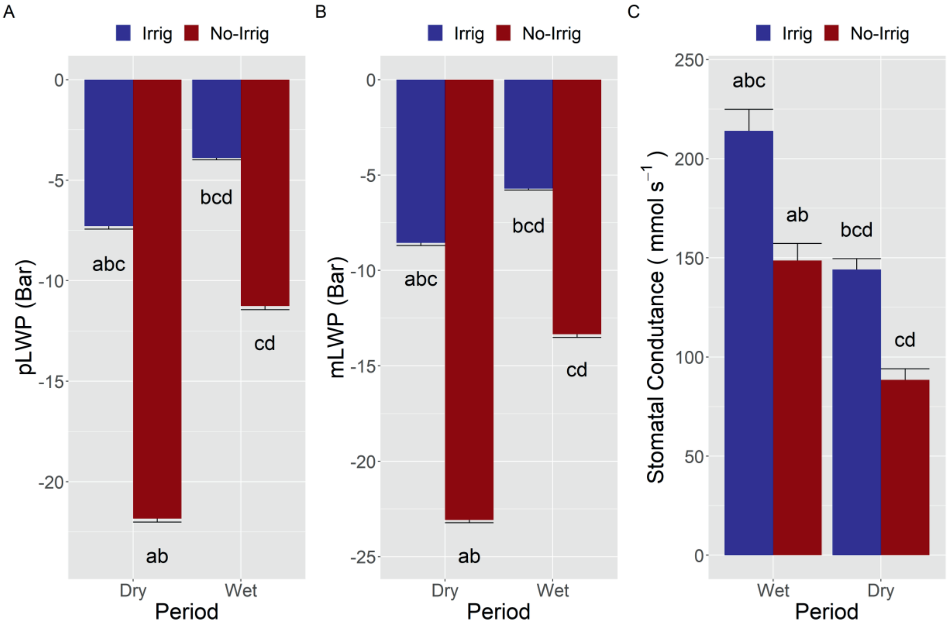


Fig. A.3.3 The effect of irrigation, period (dry vs. wet seasons) and their interaction on leaf physiological traits (A) Predawn leaf water potential (pLWP, Bar), (B) Midday leaf water potential (mLWP, Bar), (C) Stomatal conductance (mmol s^{-1}). in irrigated treatment (Irrig) and no irrigation treatment (No-Irrig). Letters above and below the error bars indicate the Compact Letter Display (cld) of the statistically significant different groups after a Tukey HSD Post-hoc test for multiple pairwise comparisons of the groups.

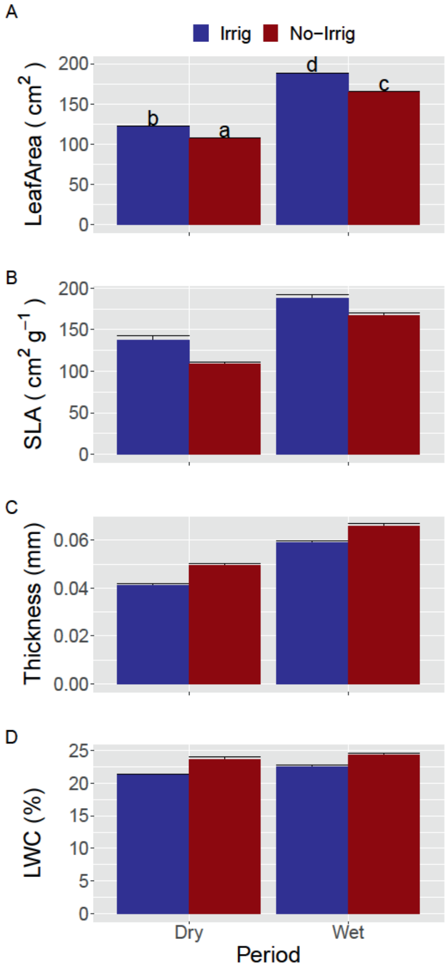


Fig. A.3.2 The effect of irrigation, measurement period (dry vs. wet seasons) and their interaction on cocoa leaf morphological traits: (A) Leaf area (cm^2), (B) Specific leaf area (SLA, cm^2 g^{-1}), (C) Leaf thickness (mm), (D) leaf water content (LWC, %) for each of the six genotypes in the irrigated treatment (Irrig) and no-irrigation treatment (No-Irrig). Errors bars represent standard errors. Tukey PostHoc letters represent significant interaction effect between irrigation, period (Table A.3). Letters above the error bars indicate the Compact Letter Display (cld) of the statistically significant different groups after a Tukey HSD Post-hoc test for multiple pairwise comparisons of the groups.

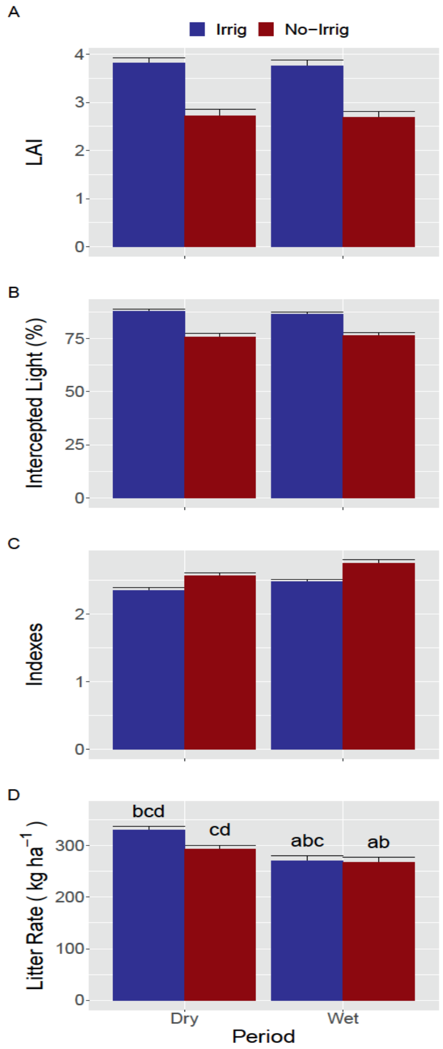
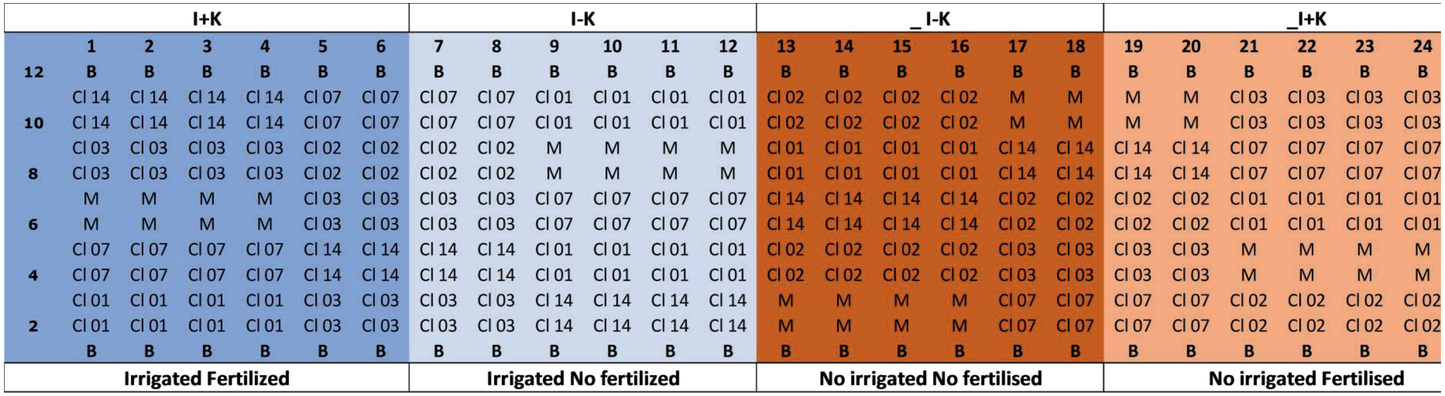


Fig. A.3.3 shows the effect of irrigation, measurement period (dry vs. wet) and their interaction on cocoa (A) leaf area index changes, (B) Percentage of light intercepted (%) by the crown, (C) water stress index and (D) monthly litterfall (kg ha^{-1}) in the irrigated treatment (Irrig) and no-irrigated treatment (No-Irrig). Error bars represent standard errors. Tukey PostHoc letters represent significant interaction effect between irrigation, period (Table A.3). Letters above the error bars indicate the Compact Letter Display (cld) of the statistically significant different groups after a Tukey HSD Post-hoc test for multiple pairwise comparison.



	I+K						I-K						_ I-K						_I+K					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
12	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**
	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03
10	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03
	Cl 03	Cl 03	Cl 03	Cl 03	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 01	Cl 01	Cl 01	Cl 01	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07
8	Cl 03	Cl 03	Cl 03	Cl 03	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 01	Cl 01	Cl 01	Cl 01	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07
	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 02	Cl 02	Cl 02	Cl 02	Cl 01	Cl 01	Cl 01	Cl 01
6	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 02	Cl 02	Cl 02	Cl 02	Cl 01	Cl 01	Cl 01	Cl 01
	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	Cl 03	Cl 03	Cl 03	Cl 03	M	M	M	M
4	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	Cl 03	Cl 03	Cl 03	Cl 03	M	M	M	M
	Cl 01	Cl 01	Cl 01	Cl 01	Cl 03	Cl 03	Cl 03	Cl 03	Cl 14	Cl 14	Cl 14	Cl 14	M	M	M	M	Cl 07	Cl 07	Cl 07	Cl 07	Cl 02	Cl 02	Cl 02	Cl 02
2	Cl 01	Cl 01	Cl 01	Cl 01	Cl 03	Cl 03	Cl 03	Cl 03	Cl 14	Cl 14	Cl 14	Cl 14	M	M	M	M	Cl 07	Cl 07	Cl 07	Cl 07	Cl 02	Cl 02	Cl 02	Cl 02
	B	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**
	Irrigated Fertilized						**Irrigated No fertilized**						**No irrigated No fertilised**						**No irrigated Fertilised**					

Fig. A.3.4. The layout of the experiment showing how the different genotypes were incorporate into the experiment

Chapter 4

Negative effects of water deficit on cocoa tree yield are partially mitigated by irrigation and potassium application

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Abstract

Yields of most tropical crops are strongly reduced by drought, but this may be partially mitigated by irrigation and potassium application. Understanding the mechanisms regulating these relationships is essential to select crop varieties reaching high yield under environmental stress. We conducted a 2-year field experiment (2020-2022) to investigate the effects of seasonal irrigation, potassium application and their interactions on cocoa reproduction and yield, using six genotypes in Côte d'Ivoire. Potassium application increased pod number and size, contributing significantly to annual yield, but this effect was conditional to soil water availability. Similarly, irrigation, when combined with potassium application, almost doubled yield from 1.5 to 3.0 kg/tree (2000 kg ha^{-1} yr^{-1} to 4000 kg ha^{-1} yr^{-1}, respectively). This yield effect was mostly the result of positive effects of irrigation on pod number per tree and, to a lesser extent, due its positive effects on bean number per pod and bean mass. Irrigation effects on pod number were associated with increased number of cherelles whereas the larger pod number during the major harvests compared to the minor harvests was associated with lower cherelle wilt. We also found a more than two-fold genotypic difference in yield, with the genotypes CI02 and CI03 having lower yields than the genotypes CI01. These genotypic yield differences were associated with differences in both cherelle wilt and initial cherelle production rates. The effects of withholding irrigation on yield were significantly dependent on the genotype, reflecting a potential genotypic difference in drought tolerance. The development of climate adaptive strategies for cocoa production requires integrating effects of irrigation, potassium application and cocoa genotype on yields. Future research should focus on unraveling the underlying genotypic and ecophysiological mechanisms of the results presented here, and identifying other potential approaches to enhance the resilience of cocoa to increasing water deficit under climate change.

Keywords: Cocoa, potassium application, irrigation, water deficit mitigation, yield

4.1 Introduction

More than 70% of world cocoa is produced in West Africa. Nearly two million small-scale farmers cultivate this crop across an estimated six million hectare of forested lands, with Côte d'Ivoire being the largest producer with 800,000 smallholder farmers and about 2 million hectares of land (Wessel & Quist-Wessel, 2015; Schulte et al., 2020; ICCO, 2021; CIFOR-ICRAF, 2021). Cocoa yield is susceptible to variable and reduced rainfall patterns (Balasimha et al. 1988; de Almeida and Valle, 2007; Schroth et al., 2016). For instance, model simulations identified that dry-season rainfall and temperature accounted for 70% of the variation in simulated cocoa yields (Zuidema et al., 2005). Hence, concerns were raised about possible yield declines in many cocoa-growing regions under climate change (ICCO, 2012), which is projected to result in more erratic rainfall especially at the drier end of the cocoa belt (Asante, 2023). In addition to its direct effects, drought can induce a higher susceptibility to pests and pathogens (CocoaNet, 2012). The aforementioned adverse effects of climate change pose significant challenges to future cocoa production (Schroth et al. 2016) worldwide. Therefore, it is crucial to develop strategies that enable cocoa farmers to cope with water deficits and ensure the sustainability of their livelihoods.

Cocoa yield is defined as being very sensitive to drought conditions as a result of rainfall reduction (Gateau-Rey et al., 2018; Bae et al., 2008). Optimal growth is achieved at annual average rainfall between 1400 and 2000 mm yr^{-1}. Growth and yield decline when annual rainfall drops below 1200 mm (Alvim, 1977) and also when dry seasons are prolonged (Zuidema, et al. 2003; Dohmen, et al. 2018). Drought stress occurs when potential water loss through transpiration exceeds the ability of the tree to absorb water, thus reducing various physiological processes and tissue growth. The most obvious agronomic measure to deal with water shortages is to apply irrigation when shortages occur (e.g., during the dry season). An old study on mature cocoa (Hutcheon, 1973) showed that irrigation during the dry season reduced cherelle wilt and increased pod setting and yield by over 40%. Similar effects were found in rain exclusion studies, resulting in a 10% decline in cocoa yield (Moser et al., 2010). So far, most studies on drought effects in cocoa have focused on seedlings and – as far as we know – few published studies have analyzed dry-season irrigation effects on yield in adult field-grown cocoa trees. Therefore, there is relatively little knowledge on the effects of dry-season irrigation on cocoa production and how these effects are modified by genotype.

An important agronomic practice in cocoa is ensuring adequate potassium availability. Cocoa requires large amounts of potassium to produce high yields (Thong & Ng, 1980). Potassium accounts for about 70% of the nutrient load in xylem sap of cocoa trees (Martins, 1976), and it is the most exported mineral in cocoa pod husks during harvest (de Almeida & Valle 2007; Hougni et al., 2021). In cocoa seedlings, potassium application (3 - 4 g plant-1) increased biomass during water deficit, promoted drought recovery survival (Kaba et al., 2022) and enhanced cocoa resilience to drought (Djan et al., 2018). These findings suggest that potassium application can help to mitigate negative effects of water deficit on cocoa seedling growth (de Almeida and Valle, 2007; Djan et al., 2018; Kaba et al., 2022), which has been documented in other crops such as cassava (Chua et al., 2020) and banana (Taulya, 2013). Yet, studies on cocoa, were limited to seedlings in greenhouses. In a previous study (Adet et al., 2024; Chapter 3), K fertilizer application and irrigation did not have any interactive effects on physiological characteristics such as stomatal conductance, leaf water potential or specific leaf area. Yet, the role of potassium application on pod formation and yield of mature cocoa trees has not been studied. Here we study the extent to which potassium availability and irrigation have interactive effects on yield and how these effects are driven by pod dynamics.

The environmental and management effects on cocoa may differ between different cocoa genotypes (Medina et al., 2017; Kaba et al., 2022). For instance, studies on drought tolerance have found that negative effects of water deficit on yield varied among cocoa genotypes (End et al. 1988; de Almeida & Valle, 2007; Daymond & Hadley, 2008; Gateau-rez et al., 2018). This genotypic variation represents a resource for developing climate-resilient genotypes that better tolerate the impacts of water deficit and maintain high productivity (Lahive et al., 2019; Raza et al., 2019; Nasser et al., 2020). Unfortunately, few published studies have analyzed genotypic difference in cocoa yield in responses to variation in dry-season irrigation and potassium application in adult trees under field conditions. We therefore study the effects of water stress on components of cocoa yield for a number of genotypes.

This study aimed to investigate the effects of irrigation, potassium application and their interaction on yield, yield components and reproductive components of adult trees of six cocoa genotypes under field conditions. We hypothesize that: (1) water deficit decreases annual yield, pod number and mass, and bean size; (2) water deficit decreases cherelle number and increases the fraction of cherelles that wilt, as well as pests and pathogens damages on pods; (3) potassium application improves annual yield, yield components and reproductive components in water deficit conditions, thus mitigating effects of drought stress, and (4) the before mentioned responses will significantly depend on genotype.

4.2 Material and Methods

4.2.1 Plant material and study Area

The experiment was performed in Côte d'Ivoire at the Nestlé R&D Research Centre in Zambakro (6°49′13.98″N, 5°16′36.26″W) located at 20 km from Yamoussoukro that has an equatorial climate comprising four seasons. The long dry season usually runs from mid-November to mid-March and is characterized by the presence, in December and January, of the 'harmattan', a dry wind from the Sahara, which considerably lowers the humidity. The long rainy season is from mid-March to mid-July, while the short dry and short wet seasons run from mid-July to mid-September and from mid-September to mid-October, respectively. Average rainfall amounts vary from 900 to 1100 mm with a highly variable temporal distribution from year to year. It ranges from 15 mm in the driest month (January) to 165 mm in the wettest one (June). Diurnal temperatures average 26 °C, with a relative humidity ranging from 75-85%, dropping to 40% during the 'harmattan' (Nov-Jan) and rising to 80-90% in the rainy period.

Somatic-embryogenesis-produced planting material for six genotypes was used for this experiment, including five clonal (CI01, CI02, CI03, CI07, and CI14) and one hybrid (Mercedes, M). The same trees, and the same genotypes, were used over the two years of the experiment. The stand, established in 2015, consisted of 240 trees (planted in a

2.5 x 3 m grid, i.e., 1320 plants ha^{-1}) cultivated in full sun. The study extended over two years (2020-2022), with N and P fertilizers applied either with or without K fertilizer. All trees received N in the form of Nitrabor (487 kg ha^{-1}) and P in the form of Di-Ammonium Phosphate (DAP, 156 kg ha^{-1}). The K-fertilized trees received KCl (207 kg ha^{-1}) with three applications in March, July, and September 2020-2021 at 0.125 kg N, 0.04 kg P, and 0.053 kg K per tree.

4.2.2 Irrigation treatments

The experiment was set up as a split-plot randomized block design with irrigation and potassium application as treatments. The cocoa stand was divided into irrigated and non-irrigated blocks, further subdivided into sub-plots being either fertilizer or non-fertilized with potassium. Irrigation was initiated in January 2020 and involved drip irrigation with a flow rate of 1 L h^{-1}. Trees were located between two emitters, spaced 80 cm apart, and received irrigation four times weekly, providing 9.3 mm day^{-1} accumulating to about 967 mm yr^{-1} applied during dry periods.

4.2.3 Yield

Yield per tree was measured by multiplying the individual dry mass of the bean (BeanMass) by the number of beans per pod (PodBeanN) and the number of pods produced per tree (PodCount) throughout different seasons over two years of production.

Yield = BeanMass x PodBeanN x PodCount Eq. (A.1)

The yield figures represent either main and small season yields or annual yields (the sum of the main and minor harvests) in kg per tree averaged over the two years of production. We present yield per tree rather than values converted to field-level values per hectare because plots were relatively small so the assumptions of a continuous field may not fully hold.

4.2.4 Cherelles dynamic and yield components

Healthy cherelles (length < 5 cm) were counted monthly and the fraction of wilted cherelles were calculated as wilted cherelles / (wilted + healthy cherelles). Two harvest seasons were defined: the main-harvest in November where pods were harvest that had developed mainly during the major wet season, and the small-harvest in April with harvested pods having mainly developed during the major dry season. The total number of ripe pods per tree (PodCount) was counted and removed during both harvests. A random subsample of 16 pods from a group of 8 trees (2 pods tree^{-1}) for each genotype was collected, pod length and fresh weight (PodMass) were determined, and the number of beans in each pod (PodBeanN) counted and weighed. Then, a random sample of 50 beans was oven dried and individually weighed to determine the average dry bean mass (BeanMass). The dynamics of the pathogens and pathogen damage (e.g., black-pod disease, *Phytophthora palmivora* and *megakarya*) that appeared during the development of the experiment and on harvested pods were registered as the percentage of pod attacked on the tree:

$$\%\ of\ Attack \quad = \frac{N°\ of\ \ pods\ affected}{N°\ of\ pods\ harvested} * 100 \quad \text{Eq. (A.2)}$$

4.2.5 Data analysis

Linear mixed-effects models (LMMs) were used to evaluate the effects of irrigation, potassium application and their interaction, and of genotype on yield and yield components. The analysis was performed in two steps. First, we tested whether there was an effect of the harvest seasons (small vs main), irrigation and their interaction on yield and yield components. The small harvest season was considered as the period in which pods developed during dry months and then irrigation was turned on. The main harvest was the period in which pods developed during wet months with irrigation turned off. We included the harvest seasons (small vs main), irrigation and the two-way interaction between irrigation and harvest seasons as fixed effects, with a random intercept per tree. In the second step, we tested the effects of irrigation, potassium application and genotype on cocoa annual yield and yield components using annual means or totals. We included irrigation, potassium application, genotype and the interaction between irrigation and potassium as fixed effects and samples as random effects. Models were compared based on Akaike's Information Criterion (AIC), selecting the most parsimonious models with the lowest AIC values indicating a better fit (Burnham & Anderson, 2002). Effects were considered significant, if bootstrapped 95% confidence intervals of the model coefficients did not overlap with zero. Marginal R-squared (R^2m) and conditional R-squared (R^2c) were calculated; R^2m indicates the variation explained by the fixed effects only, R^2c indicates variation explained by both the fixed and random effects (Nakagawa and Schielzeth, 2013).

Statistical analyses were performed using R3.1.0 software (R Core Team). LMMs were performed using the "glmmTMB" R package (Brooks et al., 2017).

4.3 Results

4.3.1 Effect of seasons and irrigation on cocoa annual yield and yield components

Results showed that irrigation and potassium treatments influenced both soil and plant water status, consequently affecting yield components (Fig. A.4.3). The details regarding the variation in microclimate and soil moisture (Fig. A.4.3) were as described in (Chapter 3).

Irrigation significantly increased yield in both the main and small harvest seasons (Fig. 4.1, Table 4.1), but the effect was stronger for the small (~77% increase) than for the main harvest (~65% increase). This positive effect extended to all yield components, particularly the number of pods per tree (PodCount) and to a somewhat lesser extent effects on average dry bean mass. Differences between main and small harvests were consistent across all components, with the main harvest showing higher values (Fig. 4.1).

Irrigation increased cherelle numbers in both seasons, especially in the main season (Fig. 4.2. A). However, irrigation increased the fraction of wilted cherelles only in the small harvest (Fig. 4.2. B) while it increased the loss due to greater percentage of attacked pods in both seasons (Fig. 4.2. C). This implies that the positive irrigation effect on pod number resulted from increased cherelle production rather than reduced cherelle loss. On the other hand, the difference in pod number between the main and small seasons was associated with both more cherelles being produced and relatively lower rates of cherelle wilt during the main season (Fig. 4.1. B, C; Fig. 4.2. B, C).

Table 4.1: Results of linear mixed effect models to explain variation in four yield variables and three reproductive variables of experimental cocoa trees. Explanatory variables are season (small vs main season, Seas), irrigation (Irrig) treatment and their interaction (:), and are marked, with "sig" when significant. Variable names: cocoa annual yield, number of pods per trees (PodCount), average pod mass (PodMass), number of beans per pod (PodBeanN), and mass of an individual bean (BeanMass), average number of cherelles (Cher), fraction of wilted cherelles (Wilt), percentage of attacked and diseased pod (Attack). Marginal R^2 (R^2_m): variation explained by fixed effects only. Conditional R^2 (R^2_c): variation explained by both fixed and random effects.

Variables Response		**Unit**	**Predictors in best model**			**R^2_m**	**R^2_c**
	Yield	kg tree^{-1}	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.06	0.17
Yield Components	PodCount	_	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.19	0.29
	PodMass	g pod^{-1}	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.13	0.16
	PodBeanN	_	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.14	0.31
	BeanMass	g bean^{-1}	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.14	0.15
Reproductive components	Cher	_	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.04	0.05
	Wilt	_	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.03	0.06
	Attack	%	Seasons ^{Sig}	Irrig ^{Sig}	Seasons:Irrig ^{Sig}	0.07	0.08

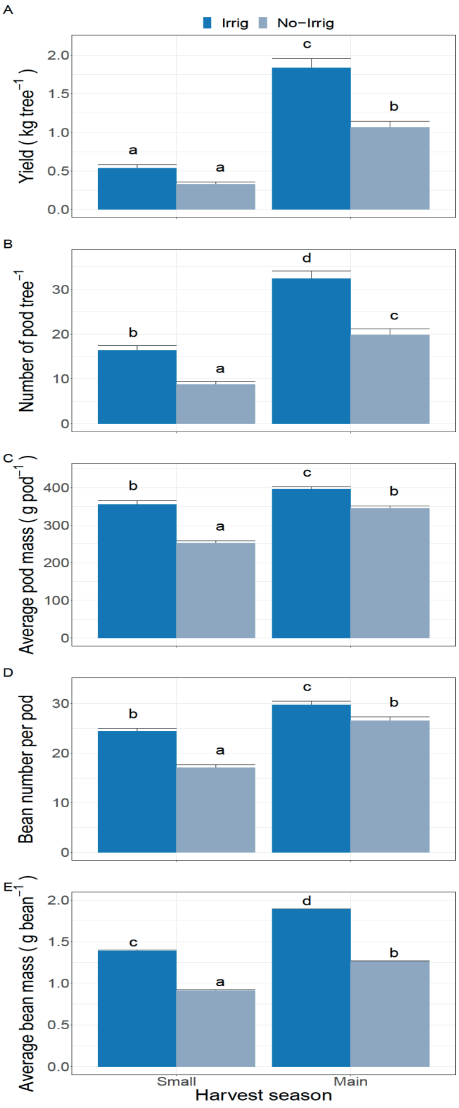

Fig. 4.1. Effects of irrigation (Irrig = irrigated vs No-Irrig=non-irrigated) and harvest season (Small vs Main) on annual cocoa yield and yield components. Mean and standard errors (whiskers) are shown for (A) annual yield per tree (B) number of pods per tree, (C) pod mass, (D) number of beans per pod and (E) individual bean mass. Pods harvested during the main harvest have developed during the wet season; those from the small harvest have developed during the dry period. Different letters above the bars indicate a significant difference.

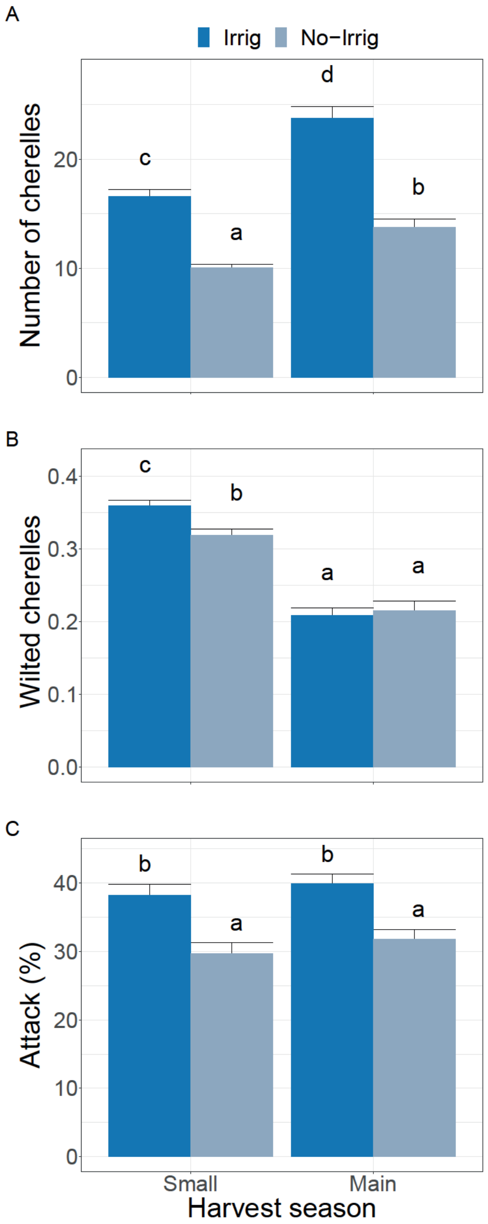

Fig. 4.2. Effects of irrigation (Irrig=irrigated; No-Irrig=non-irrigated) and harvest season (Small vs Main) on cocoa reproductive components. Mean and standard errors (whiskers) are shown for (A) Average number of cherelles (B) Fraction of wilted cherelles, (C) Percentage of attacked and diseased pods (Attack). Treatment and season effects are shown in Table 4.1. Letters are shown as in Fig. 4.1.

4.3.2 Effects of irrigation and potassium on annual yield and yield components

Annual bean yields varied between 0.5 and 3 kg tree^{-1} across treatments and seasons. Given the density of 1320 trees ha^{-1}, this converts into about 650 – 4000 kg ha^{-1} though this extrapolation needs to be taken with caution as plot sizes were relatively small. For this reason, we have chosen to report values per tree. Irrigation approximately doubled yield from ~1.5 to ~3 kg tree^{-1}, but only when K fertilizer was applied (Fig. 4.4), as indicated by the significant irrigation x K interaction (Table 4.2). This pattern was fairly consistent across the six genotypes (Fig. 4.3).

Positive irrigation and K effects on yield were observed in all yield components, especially in the number of pods per tree, which doubled from ~30 to ~60 pods when both irrigation and K were applied (Fig. 4.4. A, B-D, E). K application did not increase yield under non-irrigated conditions, but only led to more pods per tree and increased pod mass, with small effects on bean number and bean mass (Fig. 4.4. C, D, E).

Considering the reproductive dynamics, irrigation and potassium application had a large positive effect on the number of cherelles (Table 4.2; Fig. 4.5. A) mainly when K application was applied under irrigation. However, irrigation slightly increased the fraction of wilted cherelles and attacked pods (Table 4.2; Fig. 4.5. B, C). There was a significant K by irrigation interactive effect on the fraction of wilted cherelles (Table 4.2), wilting tending to slightly decline with K application in the irrigated plots but the opposite seemed to occur in the non-irrigated plots though neither trend was significant in the post-hoc analysis (Fig. 4.5. B). K application increased the percentage of attacked pods (Fig. 4.5. C). To summarize, water deficit led to fewer pod per tree, reduced cherelle wilt and resulted in smaller pods and beans. K application only stimulated pod production and yield when combined with irrigation.

Table 4.2: Results of linear mixed effect models to explain variation in four yield variables and three reproductive variables of experimental cocoa trees, as a function of genotypes (Gen, CI07, CI14, CI01, CI03, CI02, M), irrigation (Irrig), potassium application (Pot) treatment and their interaction. Significant predictors in the best model are marked with "sig". Variables names: cocoa annual yield, number of pods per trees (PodCount), average pod mass (PodMass), number of beans per pod (PodBeanN), and mass of an individual bean (BeanMass), average number of cherelles (Cher), fraction of wilted cherelles (Wilt), percentage of attacked and diseased pod (Attack). Marginal R^2 (R^2_m): model variation explained by fixed effects only. Conditional R^2 (R^2_c): model variation explained by both fixed and random effects.

Variables Responses		**Unit**	**Significant Predictors in best model**							**R^2_m**	**R^2_c**
	Annual Yield	kg tree^{-1}	Gen^{sig}	Pot^{sig}	Irrig ^{sig}	Gen:Irrig ^{sig}	Gen:Pot^{sig}	Irrig:Pot^{sig}	Gen:Irrig:Pot^{sig}	0.09	0.11
Yield Components	PodCount	_	Gen^{sig}	Pot^{sig}	Irrig ^{sig}	Gen:Irrig ^{sig}	Gen:Pot^{sig}	Irrig:Pot^{sig}	Gen:Irrig:Pot^{sig}	0.16	0.22
	PodMass	g pod^{-1}	Gen^{sig}	Pot^{sig}	Irrig ^{sig}		Gen:Pot^{sig}			0.06	0.07
	PodBeanN	_	Gen^{sig}		Irrig ^{sig}	Gen:Irrig ^{sig}				0.16	0.22
	BeanMass	g bean^{-1}	Gen^{sig}	Pot^{sig}	Irrig ^{sig}	Gen:Irrig ^{sig}	Gen:Pot^{sig}		Gen:Irrig:Pot^{sig}	0.10	0.11
Reproductive Components	Cher	_	Gen^{sig}	Pot^{sig}		Gen:Irrig ^{sig}	Gen:Pot^{sig}	Irrig:Pot^{sig}	Gen:Irrig:Pot^{sig}	0.12	0.15
	Wilt	_	Gen^{sig}	Pot^{sig}	Irrig ^{sig}	Gen:Irrig ^{sig}	Gen:Pot^{sig}	Irrig:Pot^{sig}	Gen:Irrig:Pot^{sig}	0.15	0.19
	Attack	%	Gen^{sig}	Pot^{sig}	Irrig ^{sig}	Gen:Irrig ^{sig}	Gen:Pot^{sig}			0.03	0.03

4.3.3 Genotypic effects and their interaction with irrigation and potassium application

Overall, yields differed between genotypes (Table 4.2) with CI02, and to a lesser extent CI03 exhibiting lower yield (0.3 – 1 kg tree^{-1}, depending on K and irrigation levels) compared to especially CI01 and CI14 (0.6 – 3 kg tree^{-1}). There was a significant genotype by irrigation effect on yield (Table 4.2) indicating that the effect of irrigation may differ between genotypes. While yields were consistently reduced by withholding irrigation in all genotypes this effect was significant only in CI01 (Fig. 4.3).

There was also a genotype by K interactive effect on yield. The effects of K on yields under irrigation were positive across all genotypes but only significantly so in CI01. Genotypic variation in yield was mostly associated with variation in the number of pods per tree (Fig. 4.4, Fig. A.4.1). Genotype effects on pod mass were relatively small and inconsistent. However, there were very weak genotypic differences in the response of pod mass to K as indicated by the significant K by genotype interaction (Table 4.2). Also, irrigation increased pod mass, with CI07 exhibiting the highest (384 g) and CI14 the lowest (293 g) mass (Fig. A.4.1).

Genotype effects were observed for all reproductive components (Table 4.2). The low-yielding genotype CI03 tended to produce fewer cherelles compared to other genotypes whereas the other low yielder CI02 tended to produce fewer cherelles than the other genotypes. Other genotypic differences in reproductive traits were relatively inconsistent (Fig. 4.5, Fig. A.4.2).

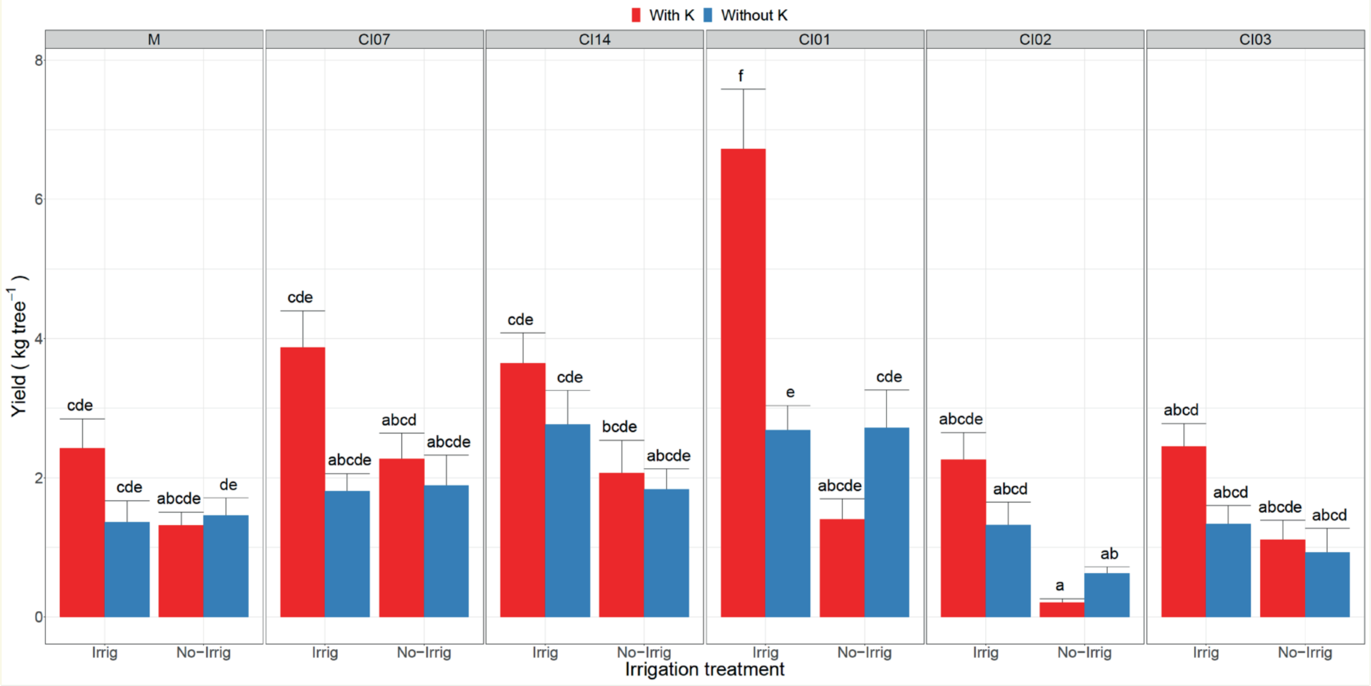

Fig. 4.3. Effects of irrigation (irrigated vs non-irrigated), potassium application (with K vs without K) on six cocoa genotypes (M, CI07, CI14, CI01, CI02, CI03) yield response. Mean and standard errors (whiskers) are shown for the annual yield per tree. Letters are shown as in Fig. 4.1.

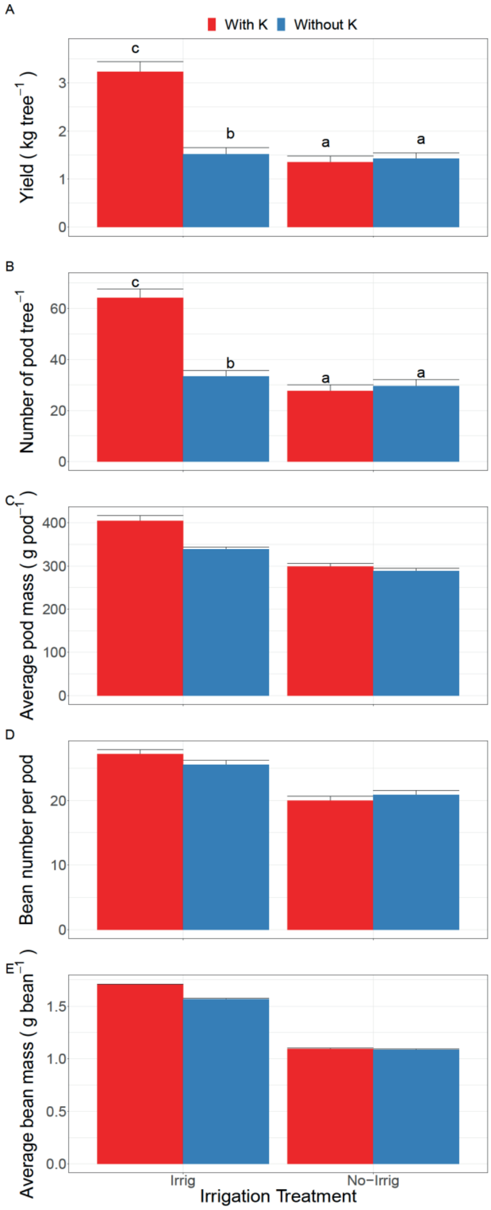

Fig. 4.4. Effects of irrigation (irrigated vs non-irrigated) and potassium application (with K vs without K) on cocoa annual yield and yield components variables. Means and standard errors (whiskers) are shown for (A) annual yield per tree (B) number of pods per tree, (C) pod mass, (D) number of beans per pod and (E) individual bean mass. Letters are shown as in Fig. 4.1.

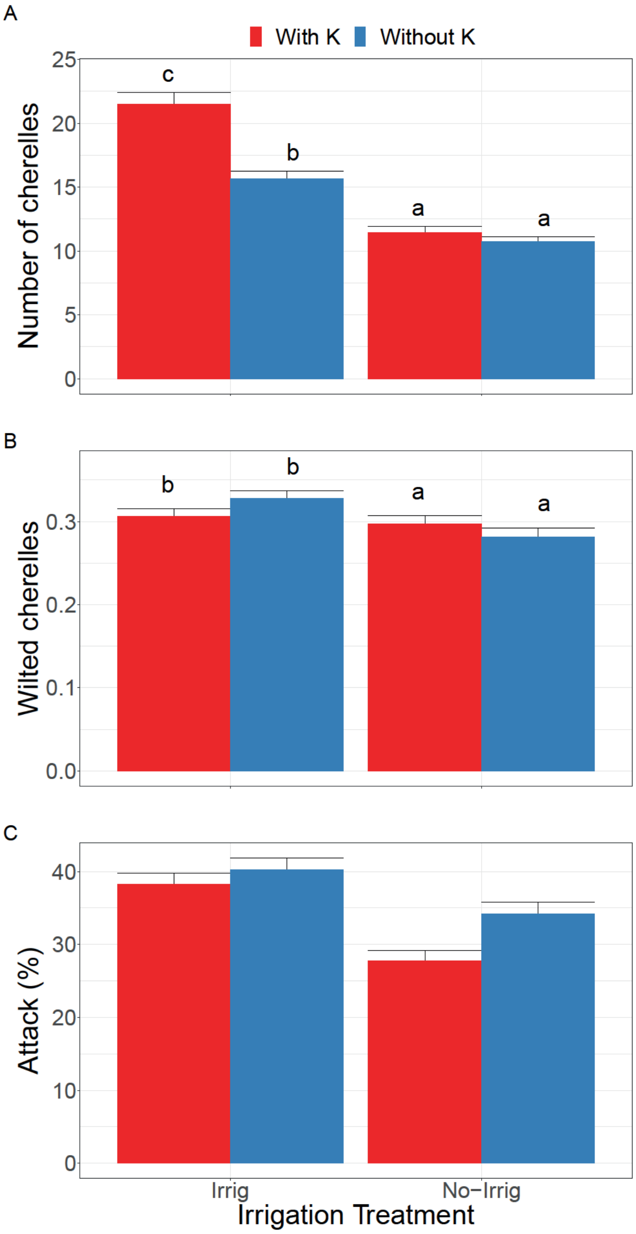

Fig. 4.5. Effects of irrigation (irrigated vs non-irrigated) and potassium application (with K vs without K) on cocoa on reproductive components. Means and standard errors (whiskers) are shown for (A) Average number of cherelles (B) Fraction of wilted cherelles, (C) Percentage of attacked and diseased pods (Attack). Letters are shown as in Fig. 4.1

4.4 Discussion

This study explored the effects of irrigation, potassium (K) application and their interaction on yield and yield components, as well as genotypic differences in these traits. Results showed significant yield responses to seasons, irrigation, K, and their interactions. Annual yield approximately doubled from ~1.5 to 3 kg tree^{-1} (roughly ~2000 to 4000 kg ha^{-1}) with application of ~960 mm dry season irrigation amounting to about 87% of the total annual rainfall (1100 mm). Irrigation mainly increased the number of mature pods and young cherelles that were produced, but also led to higher cherelle wilt incidence. K positively affected yields only under irrigation, and hence did not mitigate the negative effects of withholding irrigation on yields.

In the most favorable treatment (K fertilizer and irrigation), a three-fold difference in yield occurred across genotypes indicating the large genotypic variation in yields that exists in cocoa. There was also evidence for genotypic differences in drought tolerance, the response only being significant and also largest in CI01. These results provide significant knowledge to develop climate adaptive cocoa planting material and agronomic practices.

4.4.1 Irrigation effects on yield

Dry bean yield almost doubled from an estimated ~2000 to ~4000 kg ha^{-1} by applying 960 mm as irrigation water during the dry season albeit only when trees were also fertilized with K. This clearly indicates the importance of dry season irrigation for cocoa in relatively dry areas, like the northern part of the cocoa belt in Côte d'Ivoire, as supported by crop models emphasizing the sensitivity of cocoa yield to dry season water availability (Zuidema et al. 2005; Asante, 2023). Daymond et al., (2020) found greater seasonal variation in yields in areas with more pronounced dry seasons. This suggests that the dry season, with its associated water stress and other environmental factors, likely contributes to the increased variability in cocoa yield. Also, an experiment in Malawi revealed that more frequent irrigation treatments (1600 mm yr^{-1}) during the dry season led to a 15% yield increase compared to less frequent irrigation (920 mm yr^{-1}) (Lee, 1975). This result aligns with our finding stating that dry season irrigation (960 mm yr^{-1}) led to a two-fold increase of yield in combination with potassium. Huan et al (1986) reported a ~40% increase in pod number and annual dry bean yield (60%) due to irrigation. Our results are also consistent with the substantial yield increases documented by Diczbalis et al. (2010) under specific irrigation conditions, likely associated with differences in climate, local evapotranspiration and soil between sites. These studies show that irrigation practices can have profound positive effects on cocoa yields, emphasizing the role of water management in maximizing productivity. However, further research on irrigation effects on cocoa yields is needed to determine optimal irrigation regimes for different climate conditions considering the often existing limited availability of water resources for irrigation and the existence of competing claims on water from other uses such as the production of food crops.

The positive irrigation effect on yield was largely associated with a positive effect on the number of healthy and mature pods produced per tree and to a lesser extent with more beans per pod, and higher bean mass. However, pod mass was not affected by irrigation. These factors, identified in various studies as key yield determinants (Lachenaud, 1995; Doaré et al., 2020), show the complexity of cocoa yield responses to environmental changes. The number of pods produced often depends on environmental factors other than water availability, and is also heritable (Cilas, 1991; Cilas et al., 1999). The pod number in turn is mediated by the reproductive dynamics discussed in the next section.

4.4.2 Irrigation effects on reproductive components

Pod production is determined by the number of young pods (cherelles) produced, the fraction of cherelles that wilt (cherelle wilt) and the pods damaged by pests and diseases, particularly phytophthora. Dry season irrigation resulted in more cherelles being produced which could have been associated with a greater assimilate availability (i.e., through a greater stomatal conductance and leaf area) (Balasimha et al., 1991; Adet et al., 2024). Previous studies reported that water deficit led to greater cherelle wilt and reduced pod load in cocoa (Hadley et al., 1994; Daymond et al., 2002). The proposed explanation is that cherelle wilt is linked to a reduced assimilate supply, although the exact mechanism remains poorly understood (Valle et al., 1990; Bos et al., 2007; Melnick, 2016; Goudsmit et al., 2023). The argument is that drought conditions, through low assimilate reserves, would trigger cherelle wilt as trees have fewer resources to grow pods up to maturity. However, seemingly in contradiction with this, in our study, irrigated trees had a higher fraction of cherelle wilt. It is possible that the greater number of cherelles in the irrigated trees caused more competition for resources e.g. between larger and smaller cherelles in turn causing more of the smaller ones to wilt. Clearly, the mechanisms of assimilate allocation in cocoa trees and their impact on pod growth and cherelle wilt require more in-depth research. Particularly the question whether irrigation affects synchronicity of pod growth and its impact on cherelle wilt need more investigation, which our data resolution did not allow us to answer.

4.4.3 Effects of potassium on yield responses

K strongly increased yield, but only for irrigated trees, in line with the idea that varying a limiting production factor has a stronger effect when other factors are not limiting (Harmsen, 2000). However, this contradicts our hypothesis that K application would mitigate the negative impact of water deficit. The positive effect of K on yield can be attributed to its role in maintaining osmotic potential in the phloem, facilitating assimilate transport and enhancing stomatal functioning and photosynthesis (Cakmak, 2005; Gattward et al., 2012; Djan et al., 2018; Xu et al., 2020; Anokye et al., 2021). Our results demonstrate that applying ~200 kg ha^{-1} of K doubled annual yield and improved pod number in irrigated conditions (Fig. 4.2), consistent with previous studies showing the positive effect of K application on pod number (Lachenaud, 1991; Verlière, 1981; Uribe et al., 2001). Combined with nitrogen and phosphorus fertilization, the effect of K application on pod number is even more pronounced (Snoeck et al., 2016) consistent with our findings (note that we applied N and P fertilizers to all treatments). Results highlight that fertilizer application is more effective when other yield limiting factors – in this case water availability – are controlled for. They also emphasize that climate adaptation strategies, such as irrigation, are most effective when integrated with proper agronomic practices (Asante et al., 2021).

4.4.4 Genotypic response to irrigation, potassium and yield– tolerance

The yield varied almost three-fold across genotypes, ranging from ~0.8 kg tree^{-1} in CI03 to 3.4 kg tree^{-1} in CI01 under irrigated and K-fertilized conditions. This confirms other work (e.g., Daymond et al., 2002b) of there being strong genetic variation in yield potential in cocoa and thus there being potential for breeding. The relationship between yield and yield components also differed between genotypes. For instance, CI02 produced fewer pods than most other genotypes due to lower cherelle production, while CI03 had lower pod numbers due to a higher rate of cherelle wilt. Yield response is likely influenced by distinct physiological mechanisms regulating cherelle production and wilt as reported by (Bekele et al., 2022). Furthermore, some of the clones (notably CI01) had higher yields than the currently widely used hybrid Mercedes, indicating there is potential for yield increases through use of different genotypes than the ones currently disseminated to farmers.

There was a genotype by irrigation effect on yield indicating there was at least some degree of genotypic variation in drought tolerance in our selection. This variation may help provide a basis for breeding more climate-resilient cocoa. Yet, breeding in cocoa has focused on yield and disease resistance and relatively little work has been done to breed for climate resilience (Lahive et al. 2019). Post hoc analysis of our analysis revealed that while all genotypes exhibited yield reductions due to withholding irrigation, the effect was only significant in CI01 and this genotype also exhibited the largest absolute reduction in mean yield (> 1 kg tree^{-1}). Interestingly, in our previous study (Chapter 3), CI02 and CI03 exhibited the reductions in LAI and light capture when irrigation was withheld but this pattern was not reflected in our yield data. Overall, probably due to our limited number of replicates at genotype level, our data are insufficiently accurate to determine which genotypes are most drought tolerant. Further research, including more genotypes and environmental conditions, is necessary to develop cocoa genotypes with combined high yield and drought tolerance.

Lower attacked pod percentages in CI02 and CI03 suggest promising tolerance to biotic stress, potentially related to hormonal signals and immune responses (Ku et al., 2018; Iqbal et al., 2022). Genotypes could possess specific receptors that recognize pathogen and/or environment changes. These receptors may induce the closure of stomata on the pods' exocarp (Flores et al., 1994; Iwaro et al., 1997) and leaf epidermis, preventing pathogens from entering into the organs (Schulze-Lefert & Robatzek, 2006; Ku et al., 2018).

4.5 Conclusion

This study found that dry-season irrigation had strong positive effects on cocoa yield, but only when K was added. Thus, K application in combination with irrigation positively influences cocoa yields, potentially due to improved nutrient uptake and use. Contrary to our expectation, K did not mitigate the negative effects of water deficit on yield components. In water-limited conditions, even if sufficient K is present in the soil, trees may struggle to take it up, limiting their potential to enhance yield. Finally, we observed a very large genotypic difference in yield as well as a genotype effect on yield responses to irrigation. These findings clearly show that managing drought conditions and maximizing yield in cocoa requires a comprehensive approach that includes water management, fertilizer application and genotype selection, and overall crop management practices.

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Appendix: Effect of irrigation and potassium on each genotypes

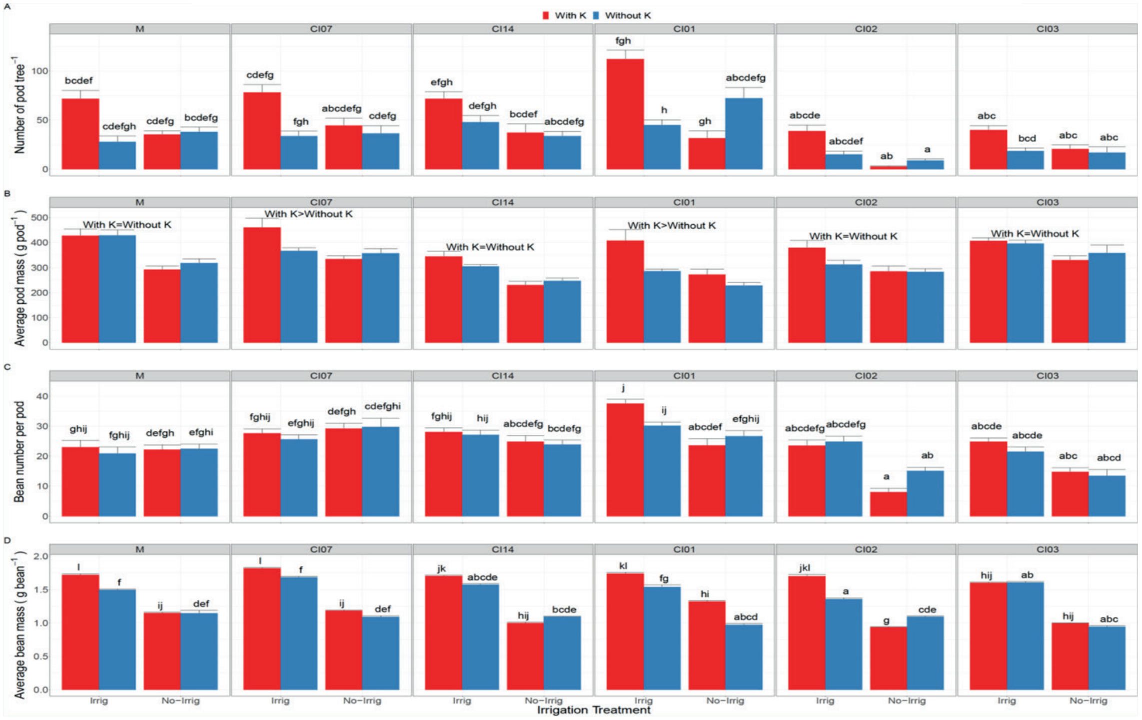

Fig. A.4.1. Effects of irrigation (irrigated vs non- irrigated) and potassium application (with K vs without K) on cocoa yield components. Mean and standard errors (whiskers) are shown for (A) number of pods per tree, (B) pod mass, (C) number of beans per pod and (D) individual bean mass. Different letters above the bars indicate a significant difference. In B, labels show the gen:Pot

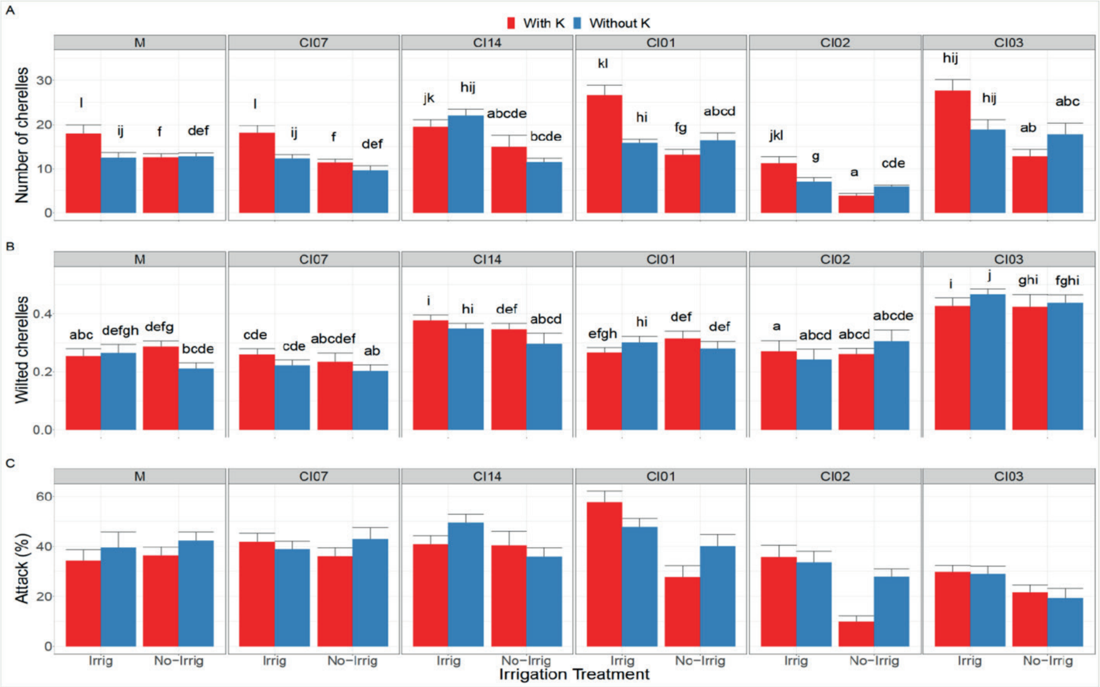

Fig. A.4.2. Effects of irrigation (irrigated vs non- irrigated) and potassium application (with K vs without K) on six cocoa genotypes (M, CI07, CI14, CI01, CI02, CI03) reproductive components. Mean and standard errors (whiskers) are shown for (A) Average number of cherelles (B) Fraction of wilted cherelles, (C) Percentage of attacked and diseased pods (Attack).

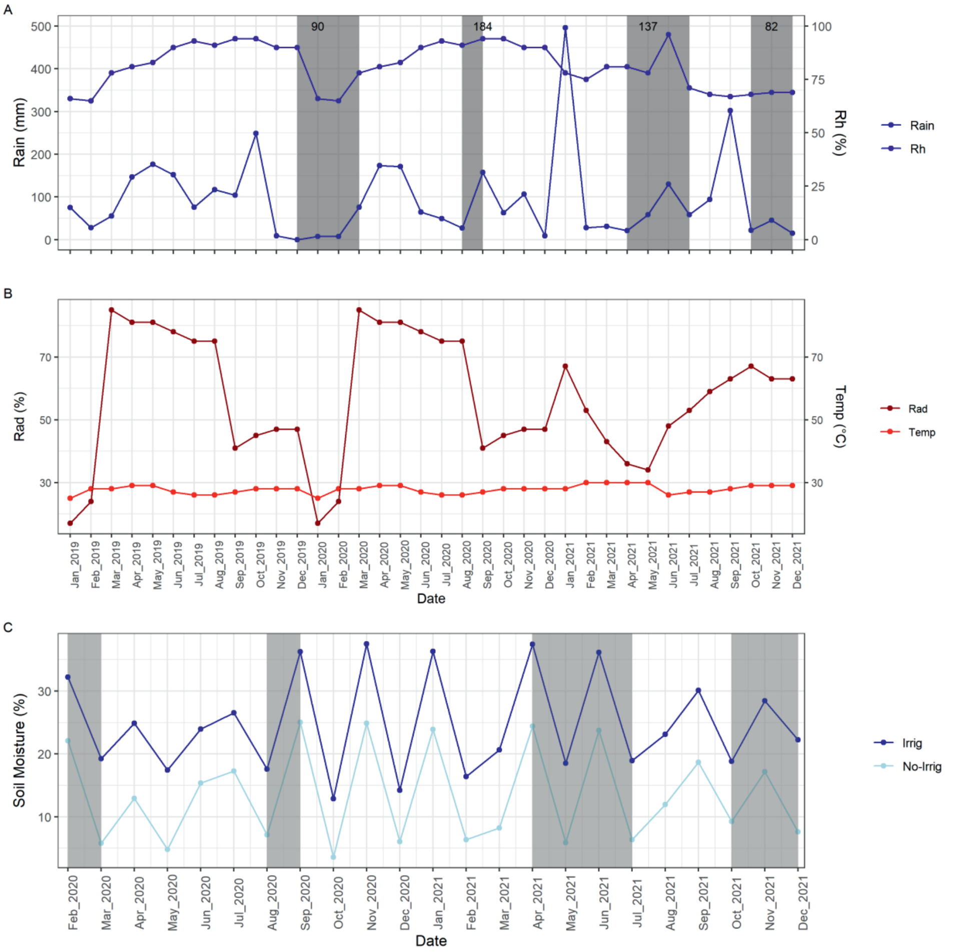

Fig. A.4.3. Monthly averages of microclimate variables throughout the experiment. (A) Rainfall (Rain; mm) on the left y-axis and relative humidity (Rh; %) on the right, (B) Solar radiation (the percentage of time with sunshine Rad; %) on the left y axis and air temperature (Temp; °C) on the right axis, (C) Soil volumetric water content (%) for irrigated (Irrig) and not irrigated (No-Irrig) plots. The grey areas indicate the dry months in which the irrigation treatment was applied; the numbers in the grey area indicate the total rainfall received in that period. Copy with permission from Adet et al., 2024.

Chapter 5

General Discussion

5.1 Challenges to cocoa production

Recent climate change predictions suggest a strong rise in climate variability over the next 100 years (IPCC, 2014) with intensifying extreme weather events, such as droughts and heat waves (IPCC, 2018). These changes are believed to negatively affect cocoa tree growth, physiology and yield (Lahive et al., 2019). Studies have shown that soil water deficits adversely affect cocoa gas exchange, i.e., leaf photosynthesis, stomatal conductance, transpiration, and overall yield (Wessel, 1971; Gattward et al., 2012; Acheampong et al., 2015; Abdulai et al., 2018a). Another study showed that water limitations resulted in significant declines in cocoa bean production (Moser et al., 2010).

The negative effects of drought on crops are primarily driven by unpredictable or at least poorly predictable factors, including rainfall patterns, soil moisture-holding capacity, and evapotranspiration rates. Droughts induce significant changes in plant hydraulic processes, assimilate partitioning, and nutrient uptake (Farooq et al., 2009). A rapid response to decreased water potential under drought is stomatal closure (Chaves et al., 2002), with stomata playing a role in regulating the balance between water loss and carbon gain - a crucial physiological process in crops. Moreover, observations reveal genetic variation in the response to water deficit tolerance among different plant varieties (de Almeida et al., 2016; Araque et al. 2012), emphasizing the importance of genetic diversity in how plants adapt to water deficits.

Drought-induced growth reductions in cocoa vary among genotypes, likely due to genotypic differences in drought adaptation and physiological responses. Studies on cocoa genotypes reveal droughts to reduce growth, leaf size, leaf life span, but these responses differ among genotypes (Joly and Hahn 1989; Deng et al., 1990b; Meinzer et al. 1992). These adaptive traits sustain plant water status by reducing transpiration (Antwi et al. 1994).

In addition to the above climate-related challenges in cocoa cultivation, there are other factors that limit cocoa productivity, including: inadequate agricultural practices, aging trees, high incidence of pests and diseases, inadequate nutrient supply, and soil fertility decline (Clough et al., 2009; Matissek et al., 2012; Wessel & Quist-Wessel, 2015;

Bunn et al., 2017). These challenges can be partially mitigated by good management practices (Asante, 2023), such as optimizing of water and nutrient-use efficiency. So far, much of our understanding of how cocoa plants respond to different combinations of water and nutrient availability and how this affects their performance is based on greenhouse experiments (Ayegboyin & Akinrinde, 2016; de Almeida et al., 2016). Insights on such responses for mature cocoa trees grown in field conditions are needed to link to the management of cocoa cultivation.

In this thesis, I therefore aimed to provide insights into the responses of mature field-grown cocoa trees to variation in soil water availability and potassium (K) application. Proper cocoa nutrition significantly influences water-use efficiency, e.g., by supporting physiological functioning of the plants. Especially K availability may also contribute to drought tolerance, thus improving tree resilience during periods of water deficit and supporting photosynthesis and yield (Verlière, 1981; van Vliet & Giller, 2017; Marschner, 2021). I experimentally manipulated water availability in two ways. The first one was by reducing the amount of rainfall that reached the soil using rainfall shelters (Chapter 2), and the second by applying dry season irrigation (Chapter 3, 4). In both experimental set ups, I explored the extent to which application of K can mitigate drought effects (Chapter 2, 3 and 4). In the irrigation experiment, I also included six different cocoa genotypes.

I aimed at answering three main questions: 1) How do reductions in the amount of rainfall reaching the soil and the application of K impact cocoa tree performance, including root length density, leaf physiology, reproduction, and yield (Chapter 2)?; 2) How do different genotypes adapt their growth and physiological responses to water deficit and K application (Chapter 3); and 3) What are the effects of water deficit and K application on the yields of different cocoa genotypes (Chapter 4)?. The current chapter brings together the key findings from Chapters 2, 3, and 4, interprets these, and formulates implications for cocoa research and cocoa cultivation. In addition, it contains some preliminary results of the effect of water availability on cocoa quality traits.

5.2 Effect of soil water availability on cocoa trees

The experiments in Chapters 2, 3, and 4 shed light on how cocoa trees respond to dry spells and on the positive impact of irrigation. Chapter 2 explored the effects of reducing the amount of rain water reaching the soil on various aspects of cocoa, including root, leaf physiological, morphological, and yield traits. This experiment was conducted to simulate a condition where less rain would fall during the rainy season. The location where this experiment was conducted (Divo) is rather wet. The reduction of water availability in this experiment mimics drought conditions that may occur under climate change (e.g., see Asante, 2023). The field irrigation experiment in Chapters 3 and 4, on the other hand, explored the effects of dry season irrigation. This experiment was conducted at a rather dry site, and the experiment thus aimed to explore the potential impact of a drought-mitigating strategy. In this present section, I review and compare the main results of these experiments. First of all, though it is essential to assess whether my treatments had their intended effect. Indeed, as intended, soil volumetric water content (VWC) was lower in the non-irrigated plots (Chapter 2) while it was reduced in the rain sheltering affecting water available to the trees (Chapter 3) and these differences in VWC persisted beyond the periods during which treatments were applied.

Rain-sheltered cocoa trees in Chapter 2 showed reduced stomatal conductance (Gs), leaf greenness, leaf area, and specific leaf area (SLA), indicating stress-induced constraints on key traits (Daymond et al., 2011). The findings align with observations from Chapter 3, which showed that irrigation positively influenced leaf water potential (LWP), stomatal conductance (Gs), sap flow density (SF), and leaf water content (LWC), as well as increasing leaf thickness in the wet period. Additionally, it led to a decrease in specific leaf area (SLA) during the dry period. Results from both experiments indicated that cocoa trees experiencing periods of reduced water supply (non-irrigated and rain-sheltered trees) reduce transpiration by stomatal closure and the production of smaller and thicker leaves with lower photosynthetic activity (Rozendaal et al., 2006; Taylor et al., 2012; Ayegboyin & Akinrinde, 2016; Monteiro et al., 2016). Moreover, at the canopy level, both non-irrigated (Chapter 3) and rain-sheltered trees (Chapter 2) demonstrated lower growth, including limited development of new leaves and branches, a lower canopy leaf area index (LAI), reduced light interception, higher water stress index (WSI), and an increased rate of litterfall.

Cocoa trees adapt to their environment by developing deeper and denser roots and smaller leaves, enhancing their water-use efficiency to thrive under conditions of water deficit, varying soil fertility, and differing irrigation supplies (Saavedra et al., 2020). It should be noted however that the conditions employed in these experiments (rainfall reduction and irrigation) might not comprehensively represent the diverse and dynamic conditions found in natural cocoa farming environments. Factors such as varying soil types, topography, and climate variabilities in real-world scenarios could influence cocoa tree responses differently than what our controlled experiments suggest. Chapter 2 revealed that rain sheltering significantly diminished root density, root length, in accordance with other studies (Kozlowski, 1992; Moser et al., 2010; Blum, 2011). This suggests an important sensitivity of cocoa root systems to reduced soil water availability. The observed response, where cocoa plants did not exhibit an expected increase in root growth under limited water conditions, contrasts with the typical plant behavior of allocating more resources to roots in water-limited environments. Previous studies in semi-humid conditions have shown that trees tend to produce more extensive root systems under such circumstances (Hauser et al., 1990; Hauser, 1993). It is possible that the rain-sheltered plants tried to adapt by growing larger roots. However, this adaptation likely did not make up for their reduced growth overall. Nonetheless, since root measurements were not conducted in Chapter 3, and in Chapter 2 soil moisture decline was associated with smaller roots. Therefore, it is hypothesized that cocoa trees actually increased their root growth when faced with water stress. To verify this, detailed studies, possibly involving destructive analysis, are required to examine how cocoa trees adjust their root development in response to limited water availability. Overall, these findings suggest that the environmental conditions in cocoa cultivation exert considerable effects on leaf traits and physiological processes (Balasimha et al., 1991), Both experiments demonstrated that cocoa is relatively sensitive to drought. This may be partially related to the typically shallow rooting system of cocoa.

Leaf physiology, plant reproduction and yield are tightly interconnected components of cocoa tree performance, all depending on availability of assimilates and hence on rates of photosynthesis and stomatal aperture. In both experiments, reduced Gs and leaf area were associated with significantly lower reproduction (flowering and cherelle number). Consequently, trees under water stress - those not receiving irrigation (non-irrigated) or grown under conditions simulating reduced rainfall (sheltered) - experienced significant reductions in their yield. Moreover, trees exhibited lower annual yield, fewer pods, fewer beans per pod in the sheltered trees (Chapter 2) and in non-irrigated trees (Chapter 4). These results indicate the overall negative effect of dry conditions on tree reproduction both in terms of the number and size of reproductive units. They are in line with studies showing that severe drought conditions may reduce bean yield by up to 62% (Keil et al., 2008). Irrigation mitigated losses by decreasing the proportion of attacked pods (pests) during both small and main harvests (Chapter 4).

When considering the effects of dry conditions on fruit abortion (cherelle wilt) our results were less straightforward. Dry conditions through rain sheltering led to increased cherelle wilt (Chapter 2) in line with previous studies (Hadley et al., 1994; Daymond et al., 2002). However, Chapter 4 revealed that withholding irrigation decreased cherelle wilt, contrasting with earlier findings. This highlights the complexity of how cocoa trees regulate pod number through cherelle wilt, influenced by nutrient availability. Reduced nutrient flow to cherelles, causing wilt, might result from lowered canopy photosynthesis (Chapter 2) or competition for nutrients by larger pods, as observed in Chapter 4. Manipulative experimentation where older pods are either or not removed or where flowers are simultaneously pollinized to create a smaller size difference between pods could help to further understand the mechanisms driving cherelle wilt.

In both experiments, variation in yield was correlated with pod number but in Chapter 4 this relationship was stronger. This correlation between yield and pod number suggests that pod number could be used as a proxy for yield. The number of pods is relatively easy to monitor (though counting pods in the canopy can be cumbersome). If the number of pods in a given developmental stage on trees at a certain moment in time indeed is a good indicator for yields obtained several months later, then this could help growers to predict their yields. This could have multiple benefits from enabling growers to assess potential future income, to helping the cocoa industry to forecast cocoa yields in their sourcing areas. Applicability of this however hinges on the extent to which our findings can be extrapolated to cocoa grown on typical farmer fields, which I discuss further below.

Both rain-sheltering and dry season irrigation had very strong effects on yield. The results from Chapter 2 indicate that variations in rainfall amounts during the rainy season have a significant effect on crop yields. Climate models project reductions in wet season rainfall for several parts of the West African cocoa belt (Asante, 2023). In this sense, my findings add to the concern raised by other studies (e.g., Schroth et al., 2016) that climate change could negatively impact cocoa production. The strong positive effect of irrigation on yield (Chapter 4) in turn suggests the potential of irrigation to mitigate these effects. However, the extent to which our findings can be extrapolated to cocoa production under typical conditions, requires considerable care. Yields recorded under adverse experimental conditions (i.e., low water and low K supply: 1300 – 2000 kg/ha) were still considerably higher than the typical yields achieved by farmers (~500 kg/ha). This is likely due to the controlled nature of the experimental setup, which minimized yield-reducing factors such as diseases and pests, which are known to strongly reduce yields in cocoa farms. This raises an important question about the applicability of our findings to average farming conditions. Are similar effects of water deficit to be expected for farmers? In a study on cocoa farms in Ghana, Asante et al (2023) found that cocoa yields were sensitive to climatic fluctuations mainly for the top 10% most productive farms. In the remaining farms, yields were much more influenced by agronomic practices, pest and disease management, or soil conditions. This suggests that when evaluating the effects of water availability on cocoa yields, it is essential to consider the diverse farming conditions.

5.3 Interactive effect between soil water availability and potassium on cocoa physiology and root traits

An important question addressed in this thesis was the extent to which water availability and K application interact in influencing different aspects of cocoa growth, reproduction and yield. This interactive effect was studied in all three core chapters, and thus in both experiments. The key hypothesis was that K application would mitigate the effects of drought on cocoa trees. However, in contrast to this expectation, the findings indicate that K application generally did not significantly counteract the effects of drought. I did find some positive effects of K application on cocoa growth (Chapter 2, Chapter 3), but these were mainly confined to the experimental treatments in which water availability was not reduced (no sheltering or applying irrigation). Overall, the effect of K on leaf traits (e.g. SLA, leaf area and leaf water content) was small especially compared to the stronger irrigation effect. Also, the effects of K on canopy LAI and light capture were rather inconsistent across genotypes (Chapter 3).

Individually, K application increased stomatal conductance (Gs) positively (Chapter 2), although this was not the case in Chapter 3, and it increased sap flow in Chapter 3. Also, K application positively affected root mass density (Chapter 2). These results suggest that K application can positively impact water uptake by cocoa trees and thus may have a positive effect on the tree water balance. But all these effects tended to be more strongly positive under higher than under lower water supply treatments. For sheltered trees (Chapter 2), the role of K in increasing Gs contrasts with the tree's isohydric (water saving) behavior under drought. In contrast, Chapter 3 showed that under irrigated conditions, K application complements the effects of water supply, contributing positively to tree performance. These findings suggest that while K is beneficial for cocoa tree physiology, its effectiveness is significantly influenced by water availability (Chapter 3). The timing of K application can indeed be an important factor to consider. The results showed that applying K increased Gs, but only for non-sheltered trees (Chapter 2). Therefore, applying K during the dry season might not be beneficial and could potentially harm the plants if water is limited. The lack of moisture can hinder their ability to utilize the nutrient effectively, leading to stress and potential damage. In the dry season, water deficit is typical and applying K, which increases Gs, may lead to increased water loss through transpiration. If the plant cannot adequately replenish the lost water due to limited soil moisture, it can exacerbate water stress.

The stimulation of root by K application (Chapter 2) may seem somewhat counterintuitive when considering the general tendency for root-to-shoot ratios to increase under low soil resource availability. Typically, plants enhance root growth to maximize resource uptake under nutrient limitations. However, as suggested by studies such as that of Cakmak et al. (1994), for certain nutrients like magnesium (Mg) and K, a reverse response can occur, where root growth diminishes when these nutrients become limiting. This unexpected behavior may be related to the roles that these nutrients play in the export of assimilates from leaves to roots. Thus, when K is limited, the reduced transport of these assimilates could lead to a decrease in root growth, despite the general expectation of increased root development under nutrient deficiency.

5.4 Interactive effect between soil water availability and potassium on cocoa reproduction and yield

The influence of K application on the number of healthy cherelles was relatively minor. In Chapter 4, the combination of K application with irrigation led to an increase in the number of cherelles, suggesting that K effectiveness in enhancing cherelle production is amplified when combined with adequate water supply (Rubiano, 2018) (Chapter 3). The effect of K on cherelle wilt was small and inconsistent. Overall these results indicated that the effect of K on reproductive dynamics were relatively minor and as with the leaf and root traits mostly notable under adequate water supply.

In Chapter 2, I found that K tended to influence yield under both water regimes. In Chapter 4, there was a positive effect only when plants were irrigated. Overall these findings support the idea that K application can significantly increase yield particularly when other factors such as water availability are not limiting. As African soils are often limited in K availability and as large amounts of K are removed during cocoa harvests, K fertilizer application is certainly a point of attention when attempting to sustainably increase yield. The question remains why was this yield-stimulating effect much more apparent at high than at low water availability. One reason may be that K needs to be dissolved in water as K+ ions in order to be taken up by the plant (Marschner & Rengel, 2011), thus making K uptake from dry soil difficult.

Moreover, the effects of K fertilizer application could have been somewhat masked by the trees' existing K reserves. This means that the cocoa trees might have relied on their stored K during periods of limited soil K availability, potentially influencing their response to the added fertilizer. This may especially have played a role in the irrigation experiment of Chapters 3 and 4, where all plots had been fertilized with K until September 2019. Understanding the dynamics of water and nutrient storage and utilization in cocoa trees is essential for accurately determining the thresholds at which trade-offs between growth and stress tolerance may occur. This is particularly pertinent in perennial crops such as cocoa where dynamics of uptake, storage and use may play out over multiple years. Such information could lead to more effective and sustainable cocoa cultivation practices, especially in the face of varying environmental conditions. In the studies included in this thesis, no soil chemistry analysis (nitrogen (N), phosphorus (P), potassium (K) and micro-nutrient) was conducted before and after the experiments. This poses significant limitations to the interpretation of results as I cannot discern potential nutrient co-limitations or identify the extent to which soils were deficient in K, though the significant yield response to K application in Chapter 4 does confirm there was at least some K limitation.

5.5 Why potassium does not mitigate the effect of drought on cocoa trees?

While K has shown some positive effects under water deficit conditions in this thesis (discussed in Chapters 2, 3, and 4), I did not find consistent evidence for K mitigating the expected negative impact of drought on cocoa trees. To be clear, with ‘mitigation’ I mean here that the negative effect of water limitation on cocoa tree performance measures such as yield would be smaller when K is applied than when it is not applied. My results indicate that K's ability to mitigate drought stress in cocoa trees varies with the tree's growth stage and size. For instance, while seedlings may respond well to K (Djan et al., 2018; Kaba et al., 2022), mature trees demonstrate different K requirements and responses to water deficit. This inconsistency raises questions when comparing my findings to those for other crops such as olive, banana and cassava where this drought-mitigating effect of K was observed (Arquero et al., 2006; Taulya, 2013; Chua et al., 2020). However, the exact reasons for these differences are not entirely clear at this point. Further research would be necessary to fully understand the variable effect of K across different stages of cocoa tree development and in comparison with other crops.

What I can say at this stage is that it is evident that K application benefits tree functioning and yield exclusively in the context of experimental treatment where water availability is not limited. The mitigating effect may therefore differ depending on the environmental conditions (Xiong et al., 2006). Soil type and soil pH have been found to mediate the effects of K; for instance, in sandy soils with low water retention, K benefits may be limited due to larger particles and increased pore spaces causing faster drainage and leaching of this soluble nutrient (Ogunniyi, 2017). Conversely, Clayey soils with a fine texture have a greater capacity to bind potassium due to their electrostatic properties, which can lead to improved K retention. However, this strong bond may also restrict the availability of K to plants, as the nutrient becomes less accessible for root uptake (Legesse et al., 2017; Kome et al., 2019). The experiments for this thesis were conducted on sandy-clay soil in Chapter 2 and on sandy-loam soil in Chapters 3 and 4. Acidic pH levels in soils can affect K absorption, and high soil salinity can hinder it as Na+ tends to replace K+, exacerbating drought stress (Ghiri et al., 2012; Liao et al., 2013). Unfortunately, I did not assess either the pH or salinity of the soils in our experiments.

K plays an important role regulating water uptake; as mentioned above, K application has been found to increase stomatal conductance potentially positively impacting photosynthesis and transpiration.

On the other hand, cocoa plants close their stomata to conserve water under drought conditions, as observed in the results of Chapters 2 and 3. This drought response on Gs may mask effects of K application (as discussed in Pessarakli, 2019). In our experiments, K was applied at the time when water availability treatments were installed. In Chapter 2, K increased root mass in high water conditions but not under drought. This suggests that while K application can potentially enhance root growth – and thus help mitigating drought effects – this benefit occurs in wet conditions. Therefore, K role in enhancing drought resilience through root expansion appears limited, as it does not appear to induce a more extensive root system in dry conditions (da Silva et al., 2011; Anokye et al., 2021). Furthermore, the choice of potassium chloride (KCl) as the primary source in our study may have influenced K-use efficiency. Different forms of K fertilizers have varying effects on plant physiology and nutrient uptake (Zhao et al., 2004; Hasanuzzaman et al., 2018). These forms include potassium nitrate (KNO_3), potassium sulfate (K_2SO_4), potassium carbonate (K_2CO_3), dipotassium hydrogen phosphate (K_2HPO_4), and sulfate of potash (SOP). While KCl is cost-effective and commonly used for many agronomic crops, other forms like K_2SO_4 and KNO_3 may be more suitable for crops sensitive to chloride ions (Kafkafi et al., 2001; Zhao et al., 2004; Snoeck et al., 2016). Our findings suggest that the effectiveness of K is context-dependent and constrained by the amount of available water in the soil. Consideration of alternative K forms and validation under field conditions are recommended to improve the applicability of findings to cocoa seedlings and trees in drought-prone areas (Anokye et al., 2021).

5.6 Contrasting Nutrient Dynamics: Long-lived Perennial Cocoa Plants vs. Short-lived Crops

This research, focusing on long-lived perennial plants such as cocoa, stands in contrast to studies on annual crops (e.g., winter wheat, maize, sugar beet) (Grzebisz et al., 2013), and short-lived perennials such as banana or cassava (Taulya, 2013; Chua et al., 2020). Cocoa's lifecycle, extending over 20 years, involves complex nutrient dynamics and physiological traits that evolve through various growth stages, setting it apart from crops with simpler and shorter cycles (Carr & Lockwood, 2011; de Almeida et al., 2016; Nomura et al., 2017; Chua et al., 2020). The extended interaction between soil, climate, and cocoa physiology introduces complexities in nutrient management, particularly K, not seen in annual or shorter-lived perennials (Vitousek, 2016). Unlike these plants, where nutrient allocation might focus on immediate reproductive efforts, cocoa trees manage and store nutrients over years, indicating that the impact of fertilization changes might unfold over an extended period.

The findings in Chapters 2, 3, and 4, highlighted the significant variability in cocoa tree responses to different environmental conditions. One aspect that warrants further investigation is the trees' potential to store resources, such as assimilates or nutrients like K, both during periods of water deficit and availability. This capability, especially important in long-lived perennials such as cocoa, may play a key role in determining their responses to fluctuations in soil resource availability and ultimately their resilience.

5.7 Genotypic differences in yield and yield responses to water availability and potassium application

In Chapter 4, there was a more than two-fold variation in yield across the six genotypes studied. In addition, especially the genotype CI01 exhibited a more than two-fold higher yield than Mercedes hybrid (M) the most widely-used selected material in Côte d'Ivoire. These findings align with previous work (Daymond et al., 2002) on genotypic yield variation and show a potential yield increase being attainable through breeding. The next question I addressed is whether there is also genotypic difference in responses to changes in water availability and associated drought tolerance. Here the answer is less straightforward. The findings in Chapter 4 revealed a significant genotype by irrigation interactive effect on yield suggesting there to be some degree of genotypic variation in drought tolerance. However, after conducting posthoc tests, these differences among genotypes were not statistically significant. It was therefore not possible to pinpoint which genotypes exhibited greater and which ones exhibited less drought tolerance in terms of yield. This could potentially be attributed to high variability within individual genotypes and replicates, or the ineffectiveness of the experimental conditions to differentiate genotypic responses.

There were some interesting genotypic differences when considering other trait responses to water availability. For instance, in Chapter 3 the hybrid M was able to maintain a significantly more positive leaf water potential under non-irrigated conditions than the clone CI03. In this same study, the genotypes CI02 and CI03 exhibited stronger reductions in LAI and canopy light capture when irrigation was withheld than the other genotypes. Other studies also indicated clonal variations in drought resistance within crops species and hybrids (Tschaplinski et al., 1998; Brignolas et al., 2000). Previous studies showed the importance of stomatal sensitivity in regulating water loss during transpiration as a key adaptation to drought stress (de Almeida & Valle, 2007; Apshara et al., 2013), contributing to the maintenance of LWC (Gupta et al., 2020).

Also in Chapter 3, the hybrid M and clone CI01 shed fewer senescent leaves compared to CI07, CI03, CI14, and CI02 under dry conditions. Our findings aligned with previous studies reporting genotypic differences in response to water deficit conditions, even observed in adult cocoa trees in the field, resulting in reduced LWP, photosynthesis, and stomatal conductance (Balasimha et al., 1991; Rada et al., 2005; Ávila-Lovera et al., 2016; de Almeida et al., 2016), along with enhanced translocation of photo-assimilates for root growth (dos Santos et al., 2014; dos Santos et al., 2016, dos Santos et al., 2023).

The absence of significant genotypic differences in drought response in terms of yield in the present study, however, indicates the necessity of a broader focus beyond genetics to enhance yield and climate resilience. This includes incorporating aspects like effective cultivation practices, soil management, and adaptation to microclimate conditions. Interestingly, despite the lack of significant drought response variation, Chapter 3 revealed significant effects of genotype, irrigation, and their interaction on leaf water potential.

A prevailing notion in plant and crop ecophysiology is the concept of growth-tolerance trade-off. A trade-off implies a situation where gaining an advantage in one aspect comes at the cost of losing something in another aspect. Thus, in crop ecophysiology a growth-tolerance trade-off means that increasing crop growth and yield might come at the expense of developing tolerance to specific stressors, and vice versa. When this holds across crop varieties it introduces an important choice for farmers: going for high yield potential but greater risk of substantial losses or going for less potential but also less risk. In long-lived crops such as cocoa this choice becomes particularly acute as risk accumulates over time. The reason to expect a growth-tolerance trade-off for water stress comes from the fact that traits that confer drought tolerance, such as increased root allocation or production of thicker tougher leaves are associated with slower growth, at least across species (Kattge et al., 2020). It is not clear however whether this trade-off also exists intra-specifically across cocoa genotypes.

In Chapter 4, I found no evidence for such a trade-off: high cocoa yields under favorable water availability did not necessarily come at the cost of reduced drought tolerance. This could be seen as a positive outcome as it suggests that breeding for higher yield would not necessarily entail reduced drought tolerance. In this regard, it is interesting to note that CI01 and CI07 achieved similar yields under non-irrigated conditions than the two low yielding genotypes, CI02 and CI03 did under irrigated conditions. Low-yielding genotypes (at least CI02), which had the lowest yield with irrigation, appeared to exhibit the strongest relative yield reduction in yield, albeit not significantly so. While our results are suggesting the possibility of combining high growth potential with climate resilience, they are based on a limited sample of only six genotypes. To definitively ascertain whether a growth-drought tolerance trade-off exists across cocoa genotypes, a broader screening involving a larger number of genotypes is certainly necessary.

5.8 Quality and flavor attributes of cocoa beans as a result of irrigation and potassium application

So far this thesis has dealt with the effects of water availability, K application and genotypes on the *quantity* of cocoa i.e. yield. But in cocoa, the *quality* of the beans beeb produced is very important as well. According to the published literature, few studies have explored the effects of soil conditions on cocoa quality traits (Amusan et al., 2005; Ajayi et al., 2010; Baah et al., 2011; Adewole et al., 2011). This is in spite of the fact that for the industry, cocoa quality and flavor are often as important as quantity. In a preliminary analysis, I therefore investigated how soil water availability (through irrigation) and K application influenced various attributes of cocoa beans, including chemical content and liquor flavor. Cocoa liquor, not to be confused with alcoholic beverages, is a key product in the cocoa industry, made by grinding roasted cocoa beans into a paste. In addition, I explored the extent to which these traits vary among the six genotypes that I used in Chapters 3 and 4. A subsample of beans (500g) was collected from each genotype and from each treatment (6 genotypes x 4 treatments) and used for the chemical analysis.

Because of the limited replications, I present these results only for discussion purposes and further research would be needed before conclusions can be drawn. During dry season, samples from the six studied cocoa genotypes underwent postharvest processing (Chapter 4; section 2) and were stored for two months prior to analysis. Three replicated samples were then analyzed for total titrable acidity, fat, and antioxidant content. The cocoa beans were sterilized 5 minutes at 121°C to eliminate salmonella risk and roasted 30 minutes at 125°C, which is an important step for the aroma's development. The cocoa beans were broken and shells were separated from the nibs then refined to obtain the cocoa liquor. Additionally, a technical tasting was conducted on the samples of cocoa liquors, employing a 5-point intensity linear scale. The 24 liquor samples (6 genotypes x 4 treatments) were evaluated by two tasters at Nestlé Research in Tours, France, by using a descriptive method (Box 5.1). Unfortunately, this analysis was not replicated. The liquors were tasted by genotype, with the control sample -I-K (non-irrigated without potassium treatment) tasted first, followed by the other three treatments tasted randomly I-K (irrigated without potassium treatment), -I+K (non-irrigated with potassium), I+K (irrigated with potassium). As the same plants were used as in Chapters 3 and Chapter 4, the description of the experimental set up is fully described in these chapters.

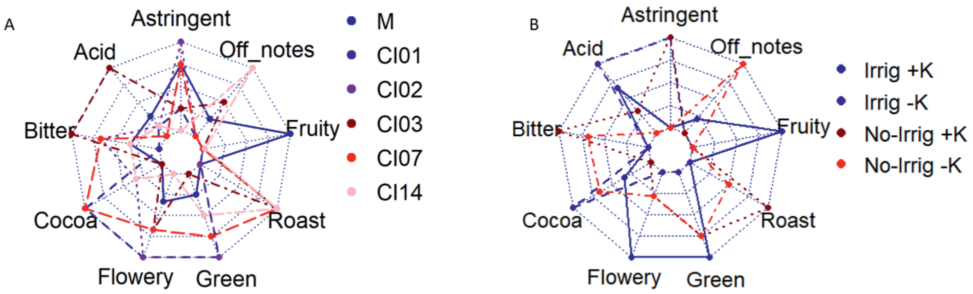

Fig. 5.4. The flavor profiles of the cocoa bean genotypes harvested during the dry season submitted to irrigation and potassium application. A: Flavor profile per genotype (M, CI01, CI02, CI03, CI07, CI14); B: Flavor profile per treatment (I+K - irrigation with potassium, -I+K - without irrigation with potassium, I-K - irrigation without potassium, -I-K - without irrigation without potassium). The flavor description is included in Box 5.1.

Attributes	Description
Acidity	Basic taste quickly perceived on the middle or on the side of the tongue that makes you salivate. Persistent flavour in mouth and can also be perceived in the throat
Bitterness	Basic taste preferably perceived and persistent on the back of the tongue/palate and throat.
Green	Flavor of raw or under-ripe fruits, or of under-fermented cocoa beans.
Cocoa	Overall flavor of well-harvested and well-processed bulk cocoa beans with no defect.
Fruity	Flavor of fresh ripe, dried or cooked fruits. e.g. apple, pear, citrus, tropical, berries, dates, raisins or figs.
Flowery	Flavour of flowers or perfumes. e.g. white flower, jasmin, rose, violet or else.
Astringent	Dry, puckering mouthfeel taste. Excessive astringency is generally undesirable, like strong tea or red wine.
Roast	Roast flavor in cocoa refers to the taste notes developed during the roasting process of cocoa beans ranging from slightly nutty and warm to deep, rich, and chocolaty.
Off-notes	Off-notes are flavors considered undesirable or atypical, fermented, moldy taste, vinegar-like.

Box 5.1: Glossary of terms and their definition as used by the cocoa liquor sensory panel

Table 5.1: Results of linear mixed-effects models evaluating effects of genotypes, potassium, irrigation and their interaction on bean chemical content in the dry season. Irrigation (Irrig), potassium application (Pot), genotypes (Gen) and their interactions were included as fixed effects and samples as random effects. All models with different combinations of fixed effects were compared using Aikaike's Information Criterion. The best model per variable is included. Bean chemical content variables were: fat content (%), antioxidant activity (antioxidant, % inhibition), and titrable acidity (meqNAOH 100g^{-1}). R^{2}m and R^{2}c refer to marginal (m) and conditional (c) R squared values. ^{Sig} indicates the significance of the fixed effects.

Variables Response		**Unit**	**Significant predictors in best model**					**R^{2}m**	**R^{2}c**
Bean Chemical Content	Fat content	%	Gen^{Sig}	Pot^{Sig}	Irrig^{Sig}	Gen:Irrig^{Sig}	Irrig:Pot^{Sig}	0.50	0.58
	Antioxydant activity	%	Gen^{Sig}	Pot^{Sig}	Irrig^{Sig}	Gen:Irrig^{Sig}	Irrig:Pot^{Sig}	0.50	0.59
	Titrable Acidity	meqNaOH 100g^{-1}	Gen^{Sig}		Irrig^{Sig}	Gen:Irrig^{Sig}		0.42	0.46

These preliminary results suggest that irrigation and K application did not alter the desirable aromatic compounds or introduced any off-flavors that could modify the overall sensory experience. Instead, the treatments appeared to maintain the beans' natural aroma profile and showed a tendency to diminish off-notes flavors. The analysis identified two important flavor notes patterns, the increase of 'flowery', considered to be a fine cocoa flavor note (Counet et al., 2004; ICCO, 2016), and the decrease of the off-notes flavor notes (Fig. 5.1. A, B). Irrigation and K application resulted in the highest flower score while no irrigation and no K addition had the lowest one. This suggests that K may positively influence this flavor note. Off-notes typically refer to undesirable or unpleasant flavors that are not part of the desired flavor profile. There was an indication that no K application and no irrigation had the highest value (Fig. 5.1. B), suggesting that application of either irrigation, K or both had a positive influence on flavor.

Concerning the chemical content, I focused on three quality traits of the cocoa bean, namely: fat content, antioxidant activity, and titrable acidity. These three traits were chosen as they represent three types of quality attributes. High fat content is favorable as it allows for the production of more cocoa butter, low titrable acidity indicates good flavor while antioxidant content reflects the added health value of cocoa.
Irrigation significantly increased fat content but only when K was also applied (Table 5.1; Fig. 5.2). K and irrigation also had an interactive effect on antioxidant levels but differences were very small. A strong antioxidant content has potential health benefits (Schinella et al., 2010; Katz et al., 2011; Rodríguez-Ramiro et al., 2011). and enhances the cocoa flavor (Kongor et al., 2016). Finally, titrable acidity increased with K application under irrigated but not under non-irrigated conditions, although the differences were quite small (Table 5.1; Fig. 5.1. B).

Together, the findings indicate that irrigation and K application can enhance the flavor and quality of cocoa beans, with varying effects across different attributes. A reduction in off-notes with these treatments points to a consistently positive impact on flavor. This offering chocolate manufacturers the opportunity to achieve a more uniform flavor profile in their products (Füllemann et al., 2022). The application of K, in particular, influenced the beans' chemical composition, notably increasing fat content and acidity in irrigated conditions and enhancing antioxidant activity in non-irrigated conditions. These changes, including increased acidity under water deficit (Fig. 5.2) suggest adaptive mechanisms to drought, that influence both flavor and nutritional value. This research aligns with previous studies highlighting the role of environmental and soil conditions in shaping the flavor and chemical profile of cocoa beans and chocolate, where traits like bitterness, astringency, and off-flavors are generally less favored (Counet et al., 2004; Afoakwa et al., 2008; Kadow et al., 2013; Boza et al., 2014; Kongor et al., 2016; Cuzzuol et al., 2023).

There was substantial genotypic variation in flavor notes (Fig. 5.1. A, Table 5.1). Among the genotypes, M (hybrid) beans scored relatively high on the fruity flavor note. CI07 (clone) beans exhibited cocoa and green notes, while CI14 (clone) beans had more roast and off-notes likely due to bacteria proliferation or too high concentration of organic acids (Jinap et al., 1994; 1995). CI01 beans had flowery, cocoa, and green notes. CI02 had an astringent, flowery, and green flavor note, while CI03 had an acid, bitter, and roast note (Fig. 5.1. A). These results indicated the difference in flavor between the genotypes, which consequently influence the cocoa liquor sensory perception, as similarly found in Ecuadorian fine and bulk cocoa flavor cultivars (Moreira et al., 2016; Rottiers et al., 2019). Moreover, the low acidity of the hybrid M indicates that at least in this respect the hybrid compares positively to the clonal genotypes. CI07, CI03 and CI01 had higher antioxidant contents whereas CI14 and CI02 had lower ones. In terms of fat content, M, CI01, CI02 and CI14 were high, while CI03 had the lowest one (Fig. 5.3). Noteworthy here is that CI01 was also the highest-yielding genotype.

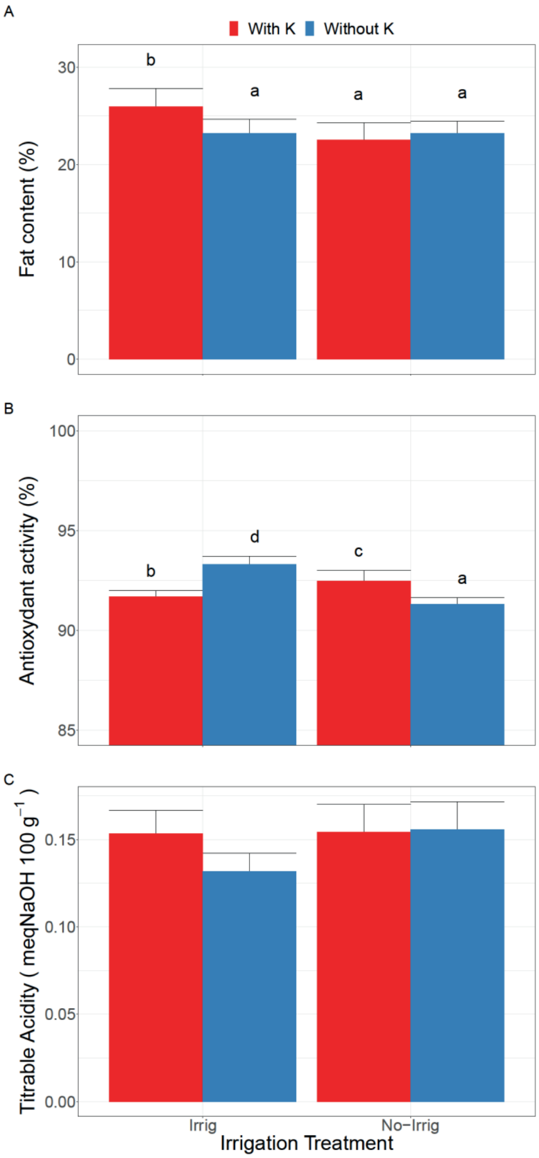

Fig. 5.5. Effects of irrigation (irrigated vs non-irrigated) and potassium application (with K vs without K) on cocoa bean chemical content parameters such as yield and yield components variables. Shown are average and standard error (whiskers) for the (A) percentage of fat content (B) antioxidant activity, and (C) titrable acidity. Significant effects of irrigation and potassium is shown in Table 5.1. Different letters above the bars indicate a significant difference.

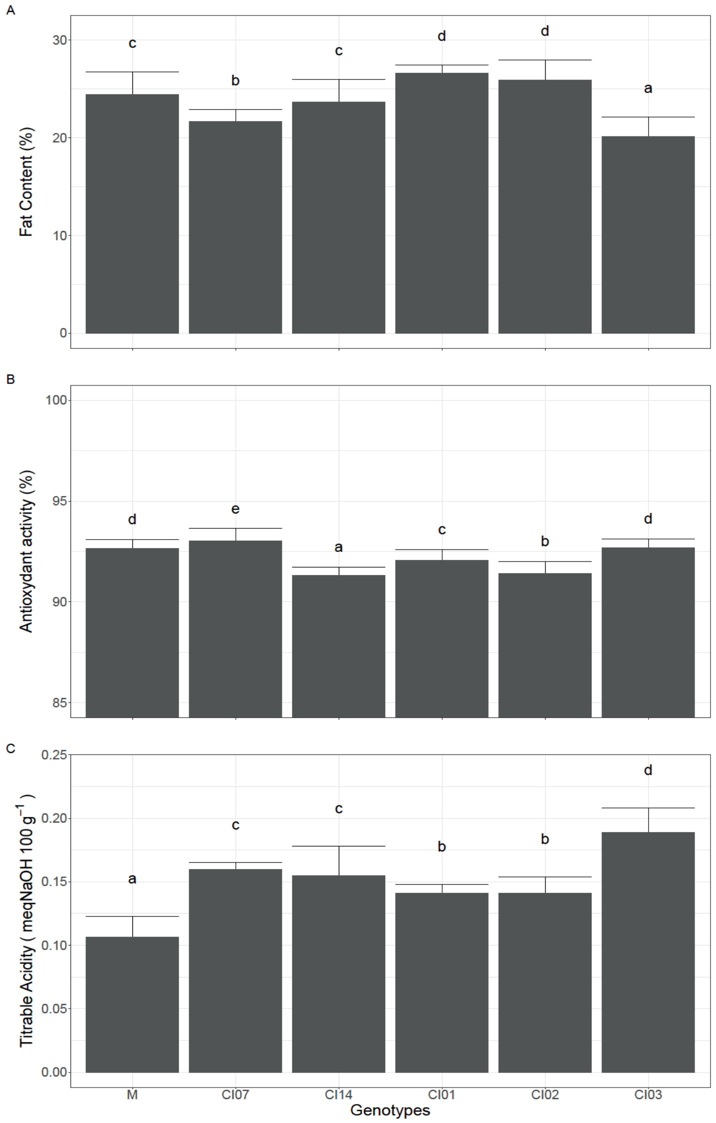

Fig. 5.6. Effects of irrigation (irrigated vs non-irrigated), potassium application (with K vs without K) on six cocoa genotypes (M, CI07, CI14, CI01, CI02, CI03) bean chemical content parameters. Data is shown as the average and standard error for the (A) percentage of fat content (B) antioxidant activity, and (C) titrable acidity. Significant effects of irrigation and potassium is shown in Table 5.1. Letters are shown as in Fig. 5.2.

The effects of genotype on the three chemical traits were influenced by the level of irrigation provided. Additionally, except for the titrable acidity, K application also played a role in these genotypic effects (Table 1). This indicates that there was a genotype by environment (GxE) effect on these quality traits as already reported by Rottiers et al. (2019). As mentioned previously, these are preliminary results and need to be established with certainty in better replicated studies. Yet, these preliminary findings suggest that breeding for quality traits in cocoa may face challenges due to genotypic responses varying with cultivation conditions. Notably, genotype comparisons revealed differences, such as the hybrid M having lower titrable acidity than others, and the clone CI03 showing the highest acidity. These variations in fat, acidity, and antioxidant levels among genotypes offer potential for creating cocoa products with distinct nutritional and flavor profiles (Counet et al.,2004; Cuzzuol et al., 2023). The strategies discussed in this thesis (irrigation and K application) collectively affect cocoa plant health and yield, as well as the chemical and quality attributes of cocoa beans.

5.9 Recommendations

My thesis does not support the concept that K application can alleviate drought effects on cocoa trees. The reduced uptake of K by plants were likely either because of the experimental plots had already received K fertilization prior to the start of the experiment, and/or the amount of K applied was too minimal or it required a longer duration to impact the plant effectively. A potential solution could be to combine slow-release K fertilizers and foliar applications to quickly deliver nutrients when soil conditions are unfavorable for root uptake (Fageria et al., 2009; Kannan, 2010; Shahena et al., 2021). While effective for micro-nutrients like Zn and Boron, this approach may be less efficient for macro-nutrients such as K, which are harder to absorb through leaves (Tiwari & Pandey, 2017; Waraich et al., 2011). However, since foliar fertilizers often include N, P, and K, they can still complement soil applications for balanced nutrition. Investigating the efficiency of cocoa leaves in absorbing nutrients through the leaves is essential for this approach to be successful. This investigation could include assessing factors such as pores at the leaf surface, the permeability of the leaf cuticle, stomata and trichomes, or hydathodes if present, along with the angle of application (Fernández & Bahamonde, 2020). Dzandu et al., (2021) found already the presence of leaf epidermal trichomes on cocoa leaves in screening for drought tolerant genotypes (Dzandu et al., 2021).

Additionally, further research should focus on investigating the pathways through which K is primarily stored in the fruits (Mengel, 2016), to enhance the understanding of K dynamics in cocoa. Currently much K is removed from the field during harvesting and more knowledge is needed to determine how much and in what way this could be replaced.

Future studies are recommended to concurrently monitor soil moisture, evapotranspiration, transpiration rate, soil water potential, and canopy temperature. This comprehensive approach will enhance the understanding of water availability for cocoa tree uptake, thereby providing valuable data to optimize K application and, when needed and feasible, to effectively schedule irrigation.

Given the preliminary nature of the current findings, it is essential to conduct more extensive and replicated research to fully understand the effects of treatment (irrigation and K application) on the chemical content and flavor of cocoa beans. The key questions revolve around profitability for farmers and affordability for consumers, making it an aspect that requires thoughtful consideration in the future.

The observed inefficiency of K to mitigate drought does not diminish the importance of K application in agriculture. Rather, it highlights the need for adapted approaches depending on specific farming conditions. For small-scale farms primarily dependent on annual rainfall, I would recommend considering additional foliar K applications during the production period to optimize nutrient uptake. In contrast, larger-scale farms, with better management capabilities and resources, might benefit from integrating both irrigation and foliar K applications to ensure consistent nutrient availability and enhanced crop productivity.

When considering management of water availability, it is clear from my results that dry-season irrigation can significantly enhance yields. When considering this option, however, it is important to ensure there are no other major limitations affecting cocoa productivity. In addition, competing claims on water resources need to be carefully considered; the question of whether, economically or in terms of food security, it is not wiser to use the water for other (agricultural) purpose, always needs to be addressed. Moreover, while irrigation presents a potential means to enhance cocoa production, its practicality and sustainability are dependent on the local environmental, economic, agronomic, and social considerations. In many parts of the cocoa growing belt it might be economically and technically feasible only for relatively affluent farmers.

Farmers should be encouraged to adopt water-saving practices such as rainwater harvesting, particularly in regions with limited water resources, to balance the demands of irrigation with the overall availability of water (Hamdy et al., 2003). In a farmer's context, rainwater harvesting involves strategically placing barrels to collect rainwater from roofs, surfaces, or trees. The collected rainwater can be stored and utilized for irrigation during periods of reduced rainfall or drought conditions (Nikolaou et al., 2020). This approach should include economic feasibility studies to ensure that it is a viable option for farmers. To overcome these challenges, it is essential to focus on research and development in sustainable irrigation and water-saving technologies, offer financial and technical assistance to farmers, enact policies promoting efficient water usage, and meticulously plan large-scale irrigation projects (Grimm and Richter, 2006; Baldwin and Stwalley, 2022). These measures should aim at not only enhancing cocoa yields but also carefully considering the broader environmental impacts and the well-being of local communities. Collaboration among governments, agricultural experts, local communities, and cocoa producers is essential to develop and implement effective, sustainable, and equitable irrigation solutions (Minh et al., 2020).

Finally, in addition to irrigation practices and K application studied in this thesis, agroforestry practices also could be ways of alleviating or buffering the impacts of climate change, including its role in exacerbating drought conditions. Integrating trees into agricultural landscapes not only enhances carbon sequestration but also contributes to biodiversity, improves soil health, and strengthens ecosystem resilience. Agroforestry has also been noted as a climate adaptive strategy, as shade trees tend to moderate daytime temperatures and reduce vapor pressure deficits. But as trees compete for water, agroforestry can also aggravate the impacts of droughts on cocoa performance and survival (Abdulai et al., 2018b). When applied along with irrigation and K application, agroforestry could provide a comprehensive solution that addresses both the immediate needs of crop production and the long-term goals of environmental sustainability.

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Summary

West Africa produces more than 70% of the global cocoa supply, Côte d'Ivoire being the largest producer, where nearly two million small-scale farmers cultivate and rely on cocoa cultivation for their livelihoods. Given that cocoa production is highly sensitive to soil water deficit, the potential impact of climate change on both cocoa yield and the economic stability of these farmers is raising major concerns. Yet, relatively little is known on the effects of water deficit and their underlying mechanisms. Nor is much known about how negative drought effects can be mitigated by irrigation, or other agronomic practices such as potassium (K) application. Finally, the extent to which genotypic variability plays a role in cocoa drought tolerance needs further exploration. This study investigated the interactions between soil water deficit, K application, and genotypic variation in cocoa plants. In chapter 2, I simulated the effects of rainfall reduction by applying rain shelters at two different levels of potassium availability. I found that rainfall shelters adversely affected cocoa root growth, leaf physiology, morphology, reproductive processes, and overall yield, underscoring the cocoa tree's susceptibility to drought. Notably, rain sheltering was observed to reduce the leaf size and crown, which in turn diminished productivity. This reduction in leaf size, a visible response to water deficit, subsequently led to decrease bean production and to lower cocoa yield. While K application had a positive effect on tree performance and yield, it could not completely counteract the detrimental effects of drought. In chapters 3 and 4, I extensively evaluated how six cocoa genotypes responded to dry-season irrigation either with or without K application in a relatively dry part of the Ivoirian cocoa producing region. The deliberate withholding of irrigation significantly impacted various cocoa traits including both traits related to biomass production (e.g. reduced stomatal conductance, leaf water potentials, leaf size, leaf area index and light capture) and reproductive traits (pod number and to a lesser extent bean number per pod). These effects were associated with a nearly two-fold difference in yield between irrigated and non-irrigated plants. This clearly indicated that at least at the drier end of the production zone, dry-season irrigation could be an effective drought mitigating

strategy. There was also a more than two-fold genotypic variation in yield indicating there being ample genetic opportunities for yield enhancement. The effects of irrigation depended significantly on genotype, emphasizing the critical need for breeding and utilizing drought-tolerant genetic cocoa material. The strategic use of irrigation during dry periods, combined with selecting drought-tolerant genotypes, could significantly bolster the resilience of cocoa production to drought. Furthermore, the research revealed that irrigation in the dry season, especially when combines with K, positively affects cocoa yield. However, contrary to our expectation, K application alone was insufficient to mitigate the negative effects of water deficit on yield components. The yield responses, which varied according to the genotype, highlighted the importance of an approach that includes genotype selection, water management, and fertilizer application to maximize cocoa yield under water-limited conditions. The study confirmed that soil water availability and potassium addition are important for cocoa trees to thrive during droughts. The knowledge acquired in this thesis could contribute to helping cocoa farmers develop strategies to drought resilient crop management strategies. However, it should also be noted that I present data obtained from research stations where conditions other than water and potassium were reasonable well controlled, and that extrapolation to farmer fields requires care. In summary, this research emphasized the urgency of adopting effective water management strategies and climate-smart agricultural practices to enhance the resilience of cocoa yield under changing climate conditions. By focusing on water conservation, appropriate fertilizer use, and the selection of suitable cocoa varieties, this work provides valuable insights into improving cocoa farming in the face of environmental challenges.

Samenvatting

West-Afrika produceert meer dan 70% van de wereldwijde cacao opbrengst, waarbij Ivoorkust de grootste producent is, waar bijna twee miljoen kleinschalige boeren cacao verbouwen en afhankelijk zijn van de cacaoteelt voor hun levensonderhoud. Aangezien de cacaoproductie zeer gevoelig is voor een tekort aan water, is de potentiële impact van klimaatverandering op zowel de cacaopbrengst als de economische stabiliteit van deze boeren zorgelijk. Toch is er relatief weinig bekend over de effecten van een tekort aan water en de onderliggende mechanismen. Ook is er niet veel bekend over hoe negatieve effecten van droogte kunnen worden verminderd door irrigatie of andere beheersmaatregelen zoals de toepassing van kalium (K). Ten slotte moet nog verder worden onderzocht in hoeverre genotypische variabiliteit een rol speelt in de droogtetolerantie van cacao. Deze studie onderzocht de interacties tussen watertekort, K-toepassing en genotypische variatie in cacaoplanten. In hoofdstuk 2 simuleerde ik de effecten van een reductie in waterbeschikbaarheid door het aanbrengen van overkappingen op twee verschillende niveaus van kaliumbeschikbaarheid. Ik vond dat overkappingen wortelgroei, bladfysiologie, morfologie, reproductieve processen en de algehele opbrengst van cacao negatief beïnvloedden, wat de gevoeligheid van de cacaoboom voor droogte benadrukt. De lagere waterbeschikbaarheid leidde tot kleinere bladeren en een kleinere kroon, wat de productiviteit verminderde. De reductie in bladgrootte, in reactie op watertekort, leidde vervolgens tot een afname in de productie van cacaobonen en dus tot een lagere cacaopbrengst. Hoewel de toepassing van K een positief effect had op de groei en opbrengst van cacaobomen, kon het niet volledig de effecten van droogte compenseren. In hoofdstukken 3 en 4 heb ik geëvalueerd hoe zes cacaogenotypen reageerden op irrigatie in het droge seizoen, met of zonder toepassing van K, in een relatief droog deel van de Ivoriaanse cacaoproducerende regio. Het achterwege laten van irrigatie had een aanzienlijke impact op verschillende cacao-eigenschappen, waaronder eigenschappen gerelateerd aan biomassaproductie (bijvoorbeeld een lager potentieel voor gaswisseling door de huidmondjes, een lagere waterpotentiaal van de bladeren, kleinere bladeren, een lagere bladoppervlakte per eenheid oppervlak en lagere opvang van licht) en reproductieve eigenschappen (aantal peulen en in mindere mate het aantal bonen per peul). Deze effecten waren geassocieerd met een bijna tweevoudig verschil in opbrengst tussen geïrrigeerde en niet-geïrrigeerde planten. Dit gaf duidelijk aan dat tenminste aan de drogere kant van de productiezone, irrigatie in het droge seizoen een effectieve strategie kan zijn om effecten van droogte te verminderen. Er was ook een meer dan tweevoudige genotypische variatie in opbrengst wat aangeeft dat er voldoende genetische mogelijkheden zijn voor opbrengstverbetering. De effecten van irrigatie waren afhankelijk van het genotype, wat het belang van het kweken en gebruiken van droogte-tolerant genetisch cacaomateriaal benadrukt. Het strategisch gebruik van irrigatie tijdens droge perioden, gecombineerd met het selecteren van droogte-tolerante genotypen, zou de veerkracht van de cacaoproductie aanzienlijk kunnen versterken. Bovendien onthulde het onderzoek dat irrigatie in het droge seizoen, vooral in combinatie met toepassing van K, een positief effect heeft op de cacaopbrengst. Echter, in tegenstelling tot onze verwachting, was de toepassing van K alleen onvoldoende om de negatieve effecten van watertekort op de cacao opbrengst teniet te doen. De variatie in de respons van verschillende cacao genotypes geeft aan dat zowel genotypeselectie, waterbeheer en bemesting van belang zijn om de cacaopbrengst lage waterbeschikbaarheid te maximaliseren. De studie bevestigde dat waterbeschikbaarheid en de toevoeging van kalium belangrijk zijn voor cacaobomen om te functioneren tijdens droogtes. De verkregen kennis in dit proefschrift zou kunnen bijdragen aan het helpen ontwikkelen van strategieën voor droogteresistente gewasbeheerstrategieën voor cacaoboeren. Ik presenteer echter resultaten die zijn verkregen op onderzoeksstations onder gecontroleerde omstandigheden; de extrapolatie naar een veldsituatie vereist zorgvuldigheid. Dit onderzoek laat de urgentie zien van het adopteren van effectieve waterbeheerstrategieën en klimaatslimme landbouwpraktijken om de veerkracht van de cacaopbrengst onder veranderende klimaatomstandigheden te verbeteren. Door de focus op waterbehoud, optimaal gebruik van meststoffen en de selectie van geschikte cacaovariëteiten, bieden de resultaten van dit onderzoek waardevolle inzichten om de cacaoteelt te verbeteren in het kader van klimaatverandering.

Résumé

L'Afrique de l'Ouest a produit plus de 70 % de l'approvisionnement mondial en cacao, la Côte d'Ivoire étant le plus grand producteur, où près de deux millions de petits agriculteurs cultivent et dépendent de la culture du cacao pour leur subsistance. Étant donné que la production de cacao est très sensible au déficit hydrique du sol, l'impact potentiel du changement climatique sur le rendement du cacao et la stabilité économique de ces agriculteurs soulève de grandes préoccupations. Pourtant, on sait relativement peu de choses sur les effets du déficit en eau et leurs mécanismes sous-jacents. On sait également peu comment les effets négatifs de la sécheresse peuvent être atténués par l'irrigation ou d'autres pratiques agronomiques telles que l'application de potassium (K). Enfin, la mesure dans laquelle la variabilité génotypique joue un rôle dans la tolérance du cacao à la sécheresse nécessite d'être davantage explorée. Cette étude a enquêté sur les interactions entre le déficit hydrique du sol, l'application de K et les variations génotypiques chez les plantes de cacao. Dans le chapitre 2, j'ai simulé les effets de la réduction des précipitations en appliquant des abris pluie à deux niveaux différents de disponibilité en potassium. J'ai constaté que les abris pluie affectaient négativement la croissance des racines du cacao, la physiologie des feuilles, la morphologie, les processus de reproduction et le rendement global, soulignant la susceptibilité du cacaoyer à la sécheresse. Notamment, l'ombrage par les abris pluie a été observé pour réduire la taille des feuilles et la couronne, ce qui à son tour diminuait la productivité. Cette réduction de la taille des feuilles, une réponse visible au déficit en eau, a par la suite conduit à diminuer la production de fèves et à réduire le rendement en cacao. Alors que l'application de K avait un effet positif sur la performance de l'arbre et le rendement, cela ne pouvait pas complètement contrer les effets néfastes de la sécheresse. Dans les chapitres 3 et 4, j'ai évalué de manière approfondie comment six génotypes de cacao répondaient à l'irrigation en saison sèche avec ou sans application de K dans une partie relativement sèche de la région productrice de cacao ivoirienne. La privation délibérée d'irrigation a eu un impact significatif sur divers traits du cacao, y compris des traits liés à la production de biomasse (par exemple, conductance stomatique réduite, potentiels hydriques des

feuilles, taille des feuilles, indice de surface foliaire et capture de la lumière) et des traits reproductifs (nombre de cabosses et dans une moindre mesure nombre de fèves par cabosse). Ces effets étaient associés à une différence de rendement presque double entre les plantes irriguées et non irriguées. Cela indiquait clairement qu'au moins à l'extrémité la plus sèche de la zone de production, l'irrigation en saison sèche pouvait être une stratégie efficace pour atténuer la sécheresse. Il y avait également une variation génotypique du rendement de plus de deux fois, indiquant qu'il y avait de larges opportunités génétiques pour l'amélioration du rendement. Les effets de l'irrigation dépendaient significativement du génotype, soulignant le besoin critique de sélectionner et d'utiliser du matériel génétique de cacao tolérant à la sécheresse. L'utilisation stratégique de l'irrigation pendant les périodes sèches, combinée avec la sélection de génotypes tolérants à la sécheresse, pourrait considérablement renforcer la résilience de la production de cacao à la sécheresse. De plus, la recherche a révélé que l'irrigation en saison sèche, surtout lorsqu'elle est combinée avec du K, affecte positivement le rendement en cacao. Cependant, contrairement à nos attentes, l'application de K seule s'est avérée insuffisante pour atténuer les effets négatifs du déficit en eau sur les composantes du rendement. Les réponses en termes de rendement, qui variaient selon le génotype, ont souligné l'importance d'une approche incluant la sélection de génotypes, la gestion de l'eau et l'application d'engrais pour maximiser le rendement du cacao dans des conditions limitées en eau. L'étude a confirmé que la disponibilité en eau du sol et l'ajout de K sont importants pour la prospérité des cacaoyers pendant les sécheresses. Les connaissances acquises dans cette thèse pourraient contribuer à aider les agriculteurs de cacao à développer des stratégies de gestion des cultures résilientes à la sécheresse. Cependant, il convient également de noter que je présente des données obtenues à partir de stations de recherche où les conditions autres que l'eau et le K étaient raisonnablement bien contrôlées, et que l'extrapolation aux champs des agriculteurs nécessite de la prudence. En résumé, cette recherche a souligné l'urgence d'adopter des stratégies de gestion de l'eau efficaces et des pratiques agricoles intelligentes face au climat pour renforcer la résilience du rendement du cacao face aux conditions climatiques changeantes. En se concentrant sur la conservation de l'eau, l'utilisation appropriée d'engrais et la

sélection de variétés de cacao adaptées, ce travail offre des perspectives précieuses pour améliorer l'agriculture du cacao face aux défis environnementaux.

Resumen

África Occidental produjo más del 70% del suministro mundial de cacao, siendo Costa de Marfil el mayor productor, donde casi dos millones de pequeños agricultores cultivan y dependen del cultivo de cacao para su sustento. Dado que la producción de cacao es muy sensible al déficit de agua en el suelo, el potencial impacto del cambio climático tanto en el rendimiento del cacao como en la estabilidad económica de estos agricultores está generando grandes preocupaciones. Sin embargo, se sabe relativamente poco sobre los efectos del déficit hídrico y sus mecanismos subyacentes. Tampoco se sabe mucho sobre cómo los efectos negativos de la sequía pueden ser mitigados por el riego o por otras prácticas agronómicas como la aplicación de potasio (K). Finalmente, en qué medida la variabilidad genotípica juega un papel en la tolerancia del cacao a la sequía necesita ser más explorada. Este estudio investigó las interacciones entre el déficit de agua del suelo, la aplicación de K y las variaciones genotípicas en las plantas de cacao. En el capítulo 2, simulé los efectos de la reducción de lluvias aplicando refugios de lluvia en dos niveles diferentes de disponibilidad de potasio. Encontré que los refugios de lluvia afectaban negativamente al crecimiento de las raíces del cacao, la fisiología y morfología de las hojas, los procesos reproductivos y el rendimiento general, destacando la susceptibilidad del cacaotero a la sequía. Notablemente, se observó que los refugios de lluvia reducían el tamaño de las hojas y la copa, lo que a su vez disminuía la productividad. Esta reducción en el tamaño de las hojas, una respuesta visible al déficit de agua, llevó a una disminución en la producción de granos y a un menor rendimiento del cacao. Aunque la aplicación de K tuvo un efecto positivo en el rendimiento y la actuación del árbol, no pudo contrarrestar completamente los efectos perjudiciales de la sequía. En los capítulos 3 y 4, evalué extensamente cómo seis genotipos de cacao respondieron al riego en la temporada seca, con o sin la aplicación de K, en una parte relativamente seca de la región productora de cacao de Costa de Marfil. La retención deliberada del riego impactó significativamente en varias características del cacao, incluyendo aquellas relacionadas con la producción de biomasa (p. ej., conductancia estomática reducida, potenciales hídricos de la hoja, tamaño de la hoja, índice de área foliar y captura de luz) y características reproductivas (número de vainas y, en menor medida, número de granos por vaina). Estos efectos se asociaron con una diferencia de casi el doble en el rendimiento entre las plantas irrigadas y no irrigadas. Esto indicó claramente que al menos en el extremo más seco de la zona de producción, la irrigación en la temporada seca podría ser una estrategia efectiva para mitigar la sequía. También hubo una variación genotípica de más del doble en el rendimiento, indicando que hay amplias oportunidades genéticas para mejorar el rendimiento. Los efectos del riego dependieron significativamente del genotipo, lo que enfatiza la necesidad crítica de criar y utilizar material genético de cacao tolerante a la sequía. El uso estratégico del riego durante períodos secos, combinado con la selección de genotipos tolerantes a la sequía, podría reforzar significativamente la resiliencia de la producción de cacao a la sequía. Además, la investigación reveló que el riego en la temporada seca, especialmente cuando se combina con K, afecta positivamente al rendimiento del cacao. Sin embargo, contrariamente a nuestras expectativas, la aplicación de K por sí sola fue insuficiente para mitigar los efectos negativos del déficit de agua en los componentes del rendimiento. Las respuestas de rendimiento, que variaron según el genotipo, destacaron la importancia de un enfoque que incluye la selección de genotipos, la gestión del agua y la aplicación de fertilizantes para maximizar el rendimiento del cacao en condiciones de limitación de agua. El estudio confirmó que la disponibilidad de agua en el suelo y la adición de potasio son importantes para que los árboles de cacao prosperen durante las sequías. Los conocimientos adquiridos en esta tesis podrían contribuir a ayudar a los agricultores de cacao a desarrollar estrategias de manejo de cultivos resistentes a la sequía. Sin embargo, también se debe señalar que presento datos obtenidos de estaciones de investigación donde las condiciones, aparte del agua y el potasio, estaban bastante bien controladas, y que la extrapolación a los campos de los agricultores requiere cuidado. En resumen, esta investigación enfatizó la urgencia de adoptar estrategias de gestión de agua efectivas y prácticas agrícolas inteligentes frente al clima para mejorar la resiliencia del rendimiento del cacao bajo condiciones climáticas cambiantes. Al enfocarse en la conservación del agua, el uso adecuado de fertilizantes y la selección de variedades de cacao adecuadas, este trabajo proporciona perspectivas valiosas para mejorar el cultivo de cacao frente a los desafíos ambientales.

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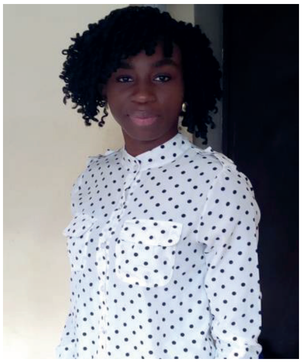
To everyone who has been part of this journey, from the bottom of my heart, thank you!

About the author



Lucette Adet was born on the 25th of July 1992 in Anyama, Côte d'Ivoire. She attended Primary, Secondary education and Bachelor with a specialization in 'Plant biology and Physiology' in Abidjan. She always loved to be out in nature and enjoyed learning new things. This led her to move to Nigeria in 2016 for her Master studies in Climate change and adapted land use. She spent one semester at the linguistic faculty School and the WASCAL (West African Science Service Centre on Climate Change and Adapted Land Use) center at the Kwame Nkrumah University of Science and Technology (KNUST) University in Ghana.

For her bachelor thesis she did a four months' internship at the West African Climate System (WACS) center, hosted by the Federal University of Technology in Akure, Nigeria, where she conducted research on the spatial patterns of sea algae distribution and the coastal population perception to climate change. She presented the results of her research at the national conference at Keffi as well as Port-Harcourt University, Nigeria. Her master thesis was in collaboration with the federal ministry of education and research (Germany), the Université de Cocody-Abidjan (Côte d'Ivoire) and The Federal University of Technology, Minna. (Nigeria).

Lucette's fascination for agriculture paved the way for her to start a PhD at the group of Crops System Analysis, department for Plant Sciences, Wageningen University of Research in the Netherlands. As a passionate Scientist, she dedicated herself to understanding and mitigating the impacts of climate change on cocoa trees, which has culminated in the completion of her PhD. This trajectory (September 2018 – May 2024) led to the thesis you hold in your hands. Lucette's work has been published in various scientific journals, and she has presented her findings at international conferences, earning recognition for her contributions to the field of Environmental Science.

When not engrossed in research exploration, Lucette enjoys dancing, sporting, and travelling.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review/project proposal (6 ECTS)

- Ecophysiology and nutrition of cocoa

Post-graduate courses (11.8 ECTS)

- Basics statistics; PE&RC (2019)
- Multivariate analysis; PE&RC (2020)
- Introduction in R and R studio; PE&RC (2020)
- Plant environmental physiology group field techniques workshop; SEB, Portugal (2022)
- Design experiments; WIAS/PE&RC (2022)
- Plant nutrients in agricultural ecosystems acquisition and turnover; PhD School of Science, University of Copenhagen (2023)
- Generalized linear models; PE&RC (2023)
- Mixed linear model; PE&RC (2023)

Competence, skills and career-oriented activities (11.65 ECTS)

- Research data management; PE&RC (2018)
- Reviewing a scientific paper; PE&RC (2019)
- Critical thinking and argumentation; PE&RC (2021)
- Scientific writing; Into'Languages (2021)
- Scientific publishing; PE&RC (2021)
- Project and time management; PE&RC (2021)
- Career orientation; WGS (2022)
- Writing propositions; PE&RC (2022)
- Effective and efficient communication in academia and beyond; PE&RC (2022)
- Presenting with impact; Into'Languages (2022)
- Proposal grant writing; Into'Languages (2023)
- Career perspectives; VLAG (2023)

Scientific integrity/ethics in science activities (0.3 ECTS)

- Ethics in plant and environmental sciences; WIMEK (2022)

PE&RC Annual meetings, seminars and PE&RC weekend/retreat (1.8 ECTS)

- PE&RC Weekend for first years (2019)
- PE&RC Last year retreat (2022)
- PE&RC Day Nijmegen (2022)

Discussion groups/local seminars or scientific meetings (14.1 ECTS)

- Journal club FEM discussion group (2018)
- R User group (2018-2020)

- 1st Cocoasoils annual meetings; Ghana (2019)
- 2nd Cocoasoils annual meetings; Cameroon (2020)
- 3rd Cocoasoils annual meetings; Online (2021)
- VTB Seminar huge tree planting projects hype or solution (2021)
- Wageningen modelling workshop (2021)
- VTB Seminar huge tree planting projects hype or solution (2021)
- Wageningen modelling workshop (2021)
- 4th Cocoasoils annual meetings; Montpellier (2022)
- 5th Cocoasoils annual meetings; the Netherlands (2023)
- 6th Cocoasoils annual meetings; Côte d'Ivoire (2024)

International symposia, workshops and conferences (7 ECTS)

- Conférence Les 1ères Journées scientifiques de l'agroforesterie; Daloa, Côte d'Ivoire (2021)
- Plant environmental physiology techniques workshop; online (2021)
- Symposium on advances in forest hydrology; Wageningen, Netherlands (2022)
- International cocoa research symposium; Montpellier, France (2022)
- INCOCOA Workshop: building research collaborations to deliver impact; Montpellier, France (2022)

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