UNIVERSIDADE FEDERAL DO CEARÁ
CENTRO DE CIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

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CONTRASTING MECHANISMS DISPLAYED BY STOMATAL CONDUCTANCE AND CO_2 FIXATION IN RESPONSE TO WATER DEFICIT AND RECOVERY IN CASHEW PLANTS

FORTALEZA
2024

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Tese apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Ceará, como parte dos requisitos para obtenção do título de Doutor (a) em Bioquímica. Área de concentração: Bioquímica Vegetal.

Orientador: Joaquim Albenísio Gomes da Silveira.

FORTALEZA

2024

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Sistema de Bibliotecas
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

S698c Sousa, Raysa Mayara de Jesus.
Contrasting mechanisms displayed by stomatal conductance and CO2 fixation in response to water deficit and recovery in cashew plants / Raysa Mayara de Jesus Sousa. – 2024.
94 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Centro de Ciências, Programa de Pós-Graduação em Bioquímica , Fortaleza, 2024.
Orientação: Prof. Dr. Joaquim Albenisio Gomes da Silveira.

1. Anacardium occidentale L.. 2. estresse hídrico. 3. reidratação. 4. regulação estomática. 5. fotossíntese. I. Título.

CDD 572

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Aprovada em: 21/11/2024.

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A Deus.

Ao melhor e maior presente da minha vida, meu filho, Dante.

Ao meu parceiro de vida e esposo, Wesley.

Aos meus preciosos e amados pais, Raimundo e Carminha.

AGRADECIMENTOS

Ao Dante, meu Dandan, que desde a barriga da mamãe contribuiu mesmo que indiretamente para que esta tese fosse realizada. Agradeço a ele toda paciência e compreensão de dividir o carinho e atenção da mamãe para que este trabalho fosse concluído. Muito obrigada meu amor!

Ao meu amado esposo Wesley, que é meu grande fã e incentivador. Agradeço-te por toda parceria, dedicação, trabalho árduo me auxiliando em vários experimentos madrugadas a fora. Você merece este título tanto quanto eu!

Aos meus pais e minha irmã: Raimundo, Carminha e Rayena que com muito orgulho ao longo desses anos têm me dado todo apoio e motivação para minha formação acadêmica.

À minha família e amigos que torceram pela concretização deste trabalho.

Ao Prof. Dr. Joaquim Albenisio Gomes da Silveira, pela orientação desde minha graduação me escolhendo como aluna de iniciação científica e me aceitando como aluna de doutoramento com total participação na formação científica que adquiri ao longo destes valorosos anos ao seu lado. Muito obrigada por me direcionar pelos caminhos da Fisiologia Vegetal.

Ao Dr. Francisco Bruno S. Freire, pela parceria, disponibilidade, orientação, sugestões na elaboração desta tese. Muito obrigada pelo seu apoio excepcional de sempre Bruno!

Ao Prof. Dr. Sérgio Luiz Ferreira-Silva, que com muita bondade nos ajudou através do empréstimo e suporte na utilização do IRGA do programa de Pós-Graduação em Produção de Plantas da Universidade Federal Rural de Pernambuco de Serra Talhada. Além das orientações, sugestões e parceria científica para produção desta tese. Muito obrigada Sérgio!

Ao professor Dr. Danilo Daloso pelos conselhos, orientações e parcerias junto ao LABPLANT. Muito obrigada prof. Danilo!

Aos queridos amigos do Laboratório de Metabolismo de Plantas (LABPLANT) Vicente, Ana Karla, Fabricio, Joãozinho, Ana Luiza, Márcio, Rachel, Humaira, Flávio, Kellyane, Rikaelly, Allana, Magna, Eva, Bruno, Valéria, Carol, Samuel, Matheus que tornaram os anos de LABPLANT e do doutoramento mais enriquecedores.

Aos amigos e parceiros de trabalho Igor Rafael, Lucas Pacheco e Alison pelas madrugadas a fio na condução de nossos experimentos compartilhando além de profissionalismo, muita alegria e boas risadas.

Aos professores, amigos e funcionários do Departamento de Bioquímica e Biologia Molecular – UFC, pelos ensinamentos e orientações, amizade e prestações de serviços que possibilitaram a realização desta tese.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

A Universidade Federal do Ceará por toda contribuição na minha formação, desde a graduação me possibilitando estar em contato com os melhores pesquisadores e professores que poderia ter.

A todas as pessoas que acreditaram em mim e torceram pelo sucesso. Muito obrigada!

RESUMO

Em decorrência das mudanças climáticas eventos adversos, tais como o aumento da incidência de déficit hídrico, principalmente em regiões mais susceptíveis tais como no semiárido brasileiro, será cada vez mais frequentes, diminuindo a produtividade agrícola e comprometendo a segurança alimentar. O cajueiro (*Anacardium occidentale* L.) é uma planta perene, sempre verde, amplamente cultivada no nordeste brasileiro e em continentes como África, Ásia, América Central, e Oceania. A produção do cajueiro se concentra principalmente em regiões intertropicais que normalmente apresentam baixa fertilidade e chuvas irregulares ao longo do ano. Neste sentido, este trabalho teve como objetivo verificar os mecanismos relacionados à condutância estomática e regulação fotossintética em plantas de cajueiro expostas a déficit hídrico. Para tanto, inicialmente a tese foi dividida em capítulo I com uma revisão sobre como plantas de cajueiro podem superar a seca extrema em ambientes semiáridos como uma planta com características de sempre verde. Já no capítulo II, foram apresentados os resultados do manuscrito em preparação, apresentando os mecanismos temporais de condutância estomática e assimilação de CO_2 em resposta à deficiência hídrica e reidratação em plantas de cajueiro. Os resultados obtidos com este manuscrito mostraram que, embora as plantas de caju não tenham apresentado alterações morfofisiológicas, a fotossíntese (A) e g_s foram drasticamente afetadas. Durante a curva de luz, plantas recuperadas (5d) não retornaram aos níveis de A e g_s observados na condição de controle. Esses parâmetros analisados durante a transição da cinética de luz e CO_2 geraram respostas distintas, mostrando que as plantas de caju podem melhorar o desempenho de A e g_s em níveis semelhantes ou superiores de condições de controle quando recuperadas. A alta sensibilidade das plantas de caju ao CO_2 é baixa, especialmente em plantas recuperadas, resultando em menor eficiência no uso da água. No geral, nossos resultados mostram que as plantas de caju têm mecanismos distintos quando sob estresses abióticos e respostas de regulação estomática.

Palavras-chave: *Anacardium occidentale* L.; estresse hídrico; reidratação; regulação estomática; fotossíntese.

ABSTRACT

Due to climate change, adverse events such as the incidence of water deficit, particularly in vulnerable regions such as in Brazilian semi-arid regions, are expected to increase and become more frequently, leading to decrease agricultural yield and food security. The cashew tree (*Anacardium occidentale* L.) is a perennial, evergreen plant widely cultivated in northeastern Brazil, as well as in continents such as Africa, Asia, Central America, and Oceania. Cashew production is primarily concentrated in intertropical regions, which are typically characterized by low soil fertility and irregular rainfall throughout the year. In this context, this study objective was to investigate the mechanisms related to stomatal conductance and photosynthetic regulation in cashew plants exposed to water deficit. To this, the thesis was initially divided into two chapters. In the chapter I, it is presented a review of how cashew plants tolerate extreme environmental conditions, such as drought in semi-arid regions, emphasizing their evergreen characteristics. I the chapter II, it is presented the manuscript in preparation' results, detailing the temporal mechanisms of stomatal conductance and CO_2 assimilation in response to water deficiency and subsequent rehydration in cashew plants. The results from this study showed that, although cashew plants did not exhibit significant morphophysiological changes, photosynthesis (A) and stomatal conductances (g_s) were severely affected. During the light curve kinetics, rehydrated plants (5 days post-stress) did not fully recover the A and g_s levels observed under control conditions. However, these parameters, assessed during the transition of light and CO_2 kinetics, exhibited distinct responses, indicating that cashew plants can enhance A and g_s g_s performance to levels comparable to or even exceeding those of control conditions upon recovery. Additionally, cashew plants demonstrated a low sensitivity to CO_2, particularly in the recovered group, resulting in reduced water-use efficiency. Overall, our findings suggest that cashew plants possess distinct mechanisms for coping with abiotic stress, including stomatal regulatory responses.

Keywords: *Anacardium occidentale* L.; water deficit; rehydration; stomatal regulation; photosynthesis.

ABBREVIATIONS LIST

$Amax$	Light saturation net CO_2 assimilation
A	Net CO_2 photosynthetic assimilation
APX	Ascorbate peroxidases
C	Control
Ca	Ambient CO_2 concentration
Ci	Intercellular CO_2 partial pressure
EL	Electrolyte Leakage
ET_o	Total plant and soil evapotranspiration
Fv/Fm	Maximum potential quantum yield of PSII
g_{max}	Maximum value for g_s under steady state
g_s	Stomatal conductance
iWUE	Intrisic water use efficiency
J_{max}	Maximum rate of photosynthetic electron transport
LCP	Light compensation point
LSP	Light saturation point
PPFD	Photosynthetically active photon flux density
REC	Recovery
RH	Relative humidity
RWC	Relative water content
Sl_{max}	Maximum slope for stomatal conductance
t50%	Time to 50% g_s variation from light transition
WW	Well-watered
Ψo	Osmotic potential
$1/\phi$	The maximum quantum efficiency of CO_2 assimilation

SUMMARY

1 INTRODUCTION

The cashew tree (Anacardium occidentale L.), a member of the Anacardiaceae family, is a perennial tropical species native to Brazil and widely cultivated in regions such as Vietnam, India, Nigeria, and Indonesia (RODRIGUES et al., 2018; FAO, 2024). Known for its economic significance, cashew production contributes substantially to the economies of these regions, with global production reaching 3.7 million tons in 2021 (FAO, 2024). Cashew plants are valued not only for their nuts but also for by-products such as cashew apple, shell liquid, and kernel, which are integral to various industries (DENDENA; CORSI, 2014; SIERRA-BAQUERO et al., 2024). Despite its economic importance, cashew cultivation faces significant challenges, particularly in semi-arid regions where water deficit, high temperatures, and soil salinity are some examples of prevalent abiotic factors (BEZERRA et al., 2007; BEGIZEW, 2021).

Cashew plants, in particular, demonstrate remarkable adaptability to drought, high temperatures, and salinity, which are common in semi-arid regions (FERREIRA-SILVA et al., 2011; LIMA et al., 2018). These adaptations are thought to involve a combination of genetic, molecular, and physiological adjustments, including the activation of antioxidant defense systems and the regulation of stomatal conductance (FERREIRA-SILVA et al., 2011; CAPELARI et al., 2021). Recent research has highlighted the role of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), in mitigating oxidative stress induced by abiotic stressors like drought and salinity (FERREIRA-SILVA et al., 2011; SUN et al., 2020). Additionally, transcriptomic studies have revealed that cashew plants upregulate genes associated with heat shock proteins (HSPs) and proline biosynthesis under water deficit conditions, further underscoring their adaptive capacity (CAPELARI et al., 2021; STEFANSKI et al., 2023).

Drought is a major abiotic stressor that poses significant challenges to plant productivity, especially in semi-arid regions (AYYAZ et al., 2021). Plants have evolved diverse strategies to cope with water scarcity, including drought avoidance, tolerance, escape, and recovery mechanisms (FANG; XIONG, 2015). Among these, stomatal regulation is a critical component of drought tolerance, as it directly influences WUE—the ratio of carbon assimilation to water transpired (PETERS et al., 2018).

As microscopic pores on plant surfaces, stomata are an evolutionary marvel that plays a pivotal role in plants' adaptation to terrestrial environments (HETHERINGTON; WOODWARD, 2003). These structures, composed of specialized guard cells, regulate the exchange of CO_2 and water vapor, balancing photosynthesis and transpiration (CLARK et al., 2022). Since their emergence in the Phanerozoic era, stomata have evolved in response to climatic changes, with their density and morphology reflecting adaptations to environmental stressors such as drought, high temperatures, and fluctuating CO_2 levels (HETHERINGTON; WOODWARD, 2003; BERRY et al., 2010).

Recent studies have highlighted the relationship between stomatal conductance (gs) and photosynthetic rates (A), and WUE as desirable components for crop improvement by optimization of photosynthetic and stomatal efficiency under water-limited conditions (EYLAND et al., 2021; MCAUSLAND et al., 2016). Environmental factors such as light intensity, CO_2 concentration, and temperature further modulate stomatal behavior, making it a complex yet essential trait for plant adaptation to changing climates (MAREK et al., 2022; LIU et al., 2023). Under drought conditions, stomatal closure is often triggered to prevent excessive water loss, which can simultaneously limit CO_2 uptake and reduce photosynthetic efficiency (Vicente-Serrano et al., 2020; Buckley, 2019).

In cashew plants, some works have demonstrated the impact of environmental conditions such as drought for gs (DE SOUZA et al., 2005; LIMA et al., 2018; CAPELARI et al., 2021). However, the mechanisms underlying stomatal recovery and photosynthetic restoration following rehydration, which is typical climate condition for semi-arid regions, remain poorly understood in cashew plants. For this reason, this study investigates the stomatal and photosynthetic responses of a cashew genotype (BRS226) to water deficit and rehydration, focusing on understanding of stomatal conductance under light and CO_2 transitions. We hypothesize that cashew plants exhibit limited sensitivity to water reestablishment, resulting in delayed restoration of photosynthetic activity and slow stomatal recovery. By characterizing these responses, we aim to shed light on the characterization in one of the unique physiological adaptations of cashew plants to semi-arid conditions, contributing to a broader understanding of drought tolerance mechanisms in crops.

Our findings may inform strategies for improving WUE and resilience in cashew and other drought-adapted species, particularly in the context of climate change.

2 OBJECTIVES

2.1 General

This thesis has the general objective of evaluating how physiological mechanisms are regulated to deal with isolated and combined abiotic stress in order to understand specific and systemic responses behind the cashew plant’s plasticity.

2.2 Specifics

1. To evaluate the isolated and combined effects of high temperature, water deficit, and salinity on cashew plants;

2. To assess biochemical, especially the antioxidant responses of cashew plants to isolated and combined effects of high temperature, water deficit, and salinity correlating to physiological adjustments;

3. To classify which abiotic stress (high temperature, water deficit, and salinity) has a higher impact for cashew antioxidant protection;

4. To analyze cashew photosynthetic responses to progressive water deficit and recovery treatments;

5. To characterize stomatal conductance regulation under light and CO_2 transitions after water deficit and recovery treatments;

6. To augment the systemic understanding of how cashew plant responds to environmental stresses, through deep studies that support the scientific knowledge already acquired in this area.

3 HOW CAN CASHEW PLANTS TO OVERCOME EXTREME DROUGHT IN SEMIARID ENVIRONMENTS AS AN EVERGREEN CROP? A REVIEW

Cashew, a relevant crop plant from the semiarid region

The Cashew tree belongs to the Anacardiaceae family, genus Anacardium with 74 genera and at least 600 species with plants varying between 5 to 14 m, which divides into dwarf and giant species (DENDENA; CORSI, 2014; NAIR, K. P, 2021). Cashew dwarf plants reach up to 5m in height with a short juvenile phase and are normally propagated by grafting. This method results in clones, which significantly enhance the orchard density and performance (DENDENA; CORSI, 2014). Cashew leaves are green, elliptic with spiral pattern, which usually grows by 3 to 14 leaves on each terminal stem (DENDENA; CORSI, 2014). Flowers are gathered in a panicle which is predominantly constituted of male and hermaphrodite flowers with 200-1,600 flowers per panicle (DENDENA; CORSI, 2014).

Cashews (*Anacardium occidentale* L.) are a well-adapted plant species in many regions worldwide, principally at Brazil, Vietnam, India, Nigeria, Indonesia, which are some of the main producers in the world (RODRIGUES *et al.,* 2018). Cashew plants are considered a perennial tropical tree from Brazil, which provides severe products and by-products such as raw and deshelled nuts, shell liquid, cashew kernel, and pseudo fruit, for instance (DENDENA; CORSI, 2014). For some countries, this agriculture commodity represents 90% of the total exports (SIERRA-BAQUERO *et al.,* 2024). In 2021, the global production of raw cashew nuts achieved 3,708,384 tons, being a recognized agricultural crop, especially in tropical areas, where this specie is extensively cultivated (FAO, 2024).

In Brazil, cashew production is of high socioeconomic importance, especially in the states from the northeast, which have the most expressive yield areas (BRITO DE FIGUEIRÊDO *et al.,* 2016). However, cashew production in this region usually faces multiple limited conditions, such as water deficit, high light, temperature, and salinity (BEZERRA *et al.,* 2007). These are some of the limited factors in semi-arid regions, that directly impacts cashew yield (BEZERRA *et al.,* 2007).

Is cashew a resilient evergreen crop for sustainable cultivation?

Evergreen plants usually activity a long life-leaf span, however, these plants can include structural differences in leaf mass and area, mechanical properties such as endurance, leaf composition, lower rates of maximum photosynthesis compared to deciduous species (WYKA; OLEKSYN, 2014). These plants usually have a higher nutrient-use efficiency (NUE) than deciduous species, low maximum photosynthetic rates, and higher lignin content (AERTS, 1995). Evergreen plants retain a full canopy all year declining their fullness during dry or winter season is less than 10% (EAMUS, 1999).

Cashew plants display great growth performance even when cultivated under limited conditions, such as those observed in semi-arid regions. These responses mostly originated from genetic, molecular, and physiological adjustments (FERREIRA-SILVA et al., 2011). A suggestion for explaining this adaptation process is that cashew plants posse physiological characteristics of evergreen plants due to its ability to growth and survive under extreme environmental conditions (LIMA *et al.,* 2018). Cashew plants are commonly cited as species belonging to the evergreen group (JOHNSTON, 2007; DENDENA; CORSI, 2014; RODRIGUES *et al.,* 2018; ADIGA *et al.,* 2020), although dendrological, taxonomic, phonological, morphological, and ecophysiological studies are scarce to classify cashew plants into this group.

One characteristic that usually includes cashew plants as part of an evergreen group in the literature is the capacity to maintain photosynthetic activity under extreme environmental circumstances (DEMMIG-ADAMS; STEWART; ADAMS, 2017). As evergreen plants, cashew plants should invest their energy to assimilate CO_2 in specific periods to achieve higher photosynthetic rates efficiency under strait opportunities (DEMMIG-ADAMS; STEWART; ADAMS, 2017). In fact, cashew plants show lower photosynthetic rates compared to other C_3 plants and crop trees (SILVEIRA *et al.,* 2003; DE SOUZA *et al.,* 2005). This mechanism might be related to unique responses of well adapted plants that in cashew plants might involve a trade-off between growth and tolerance with a personal water use strategy in face of changing environment (FERREIRA-SILVA et al., 2011) (STEFANSKI *et al.,* 2023).

As an evergreen species, cashew plants provide a stable source of food and income, particularly in tropical and subtropical under adverse conditions areas such as in semi-arid regions with high economic value as a crop plant.

Environmental conditions in the semi-arid regions of Brazil for cashew growth

Arid and semi-arid regions are present in almost half of the terrestrial surface displaying a high risk of land degradation, rainfall variability, wind erosion, and extreme heat temperatures. These are some examples of agricultural issues in these regions (BEGIZEW, 2021). The northeast of Brazil is inserted in the tropical region, where the semi-arid conditions are predominant in the majority of areas (ANDRADE *et al.,* 2016).

In these regions, the normal annual rainfall is below the minimum of most crop's water requirement, which increases the stress incidence during sensitive stages during development, affecting crop yields (BEGIZEW, 2021). In Brazil, the rainy season in the semi-arid region normally occurs in a period of 4 to 5 months, contributing to rainfall regime variability (ANDRADE *et al.,* 2016). The typical water regimes in semi-arid regions contribute to yield loss risk for crops cultivated under these conditions (BEGIZEW, 2021). One of them is caused by water stress, which is one of the most important environmental factors regulating plant growth and development *(OSAKABE et al.,* 2014).

In addition, the reduction in water availability during crop development, impact nutrient uptake, the photosynthetic machinery capacity, the related activity for enzymes related to fixation and CO_2 assimilation, and during carbon partitioning (AHANGER; SIDDIQUE; AHMAD, 2021). Cashews plants are usually rainfed cultivated, although the number of fields supplemented with irrigated systems has increased in recent years (DENDENA; CORSI, 2014). Bezerra *et al.* (2007) showed that the implementation of irrigation systems in a semi-arid region appeared to increase nut production by 70%, although this increment was not associated with higher photosynthetic rates. The fact is that unseasonal rain and drought are considered climate factors, which commonly affect cashew nuts' productivity and quality worldwide (RUPA; REJANI; BHAT, 2013).

High temperature is another agricultural limiting factor usually observed in semi-arid Brazilian regions for crop production. The increase in temperatures is a consequence of high solar energy availability in these regions, which implies higher rates in evaporative surfaces, including great evapotranspiration rates in plants (ANDRADE *et al.,* 2011). Thus, the main effects of heat consist in increase in the rate of evaporation of water, including the soil surface increasing the vulnerability to wind erosion potential, for instance (BEGIZEW, 2021). In plants, the heat stress, induced by high temperatures, causes a series of changes in plants that severely affect plant productivity (JAGADISH; WAY; SHARKEY, 2021). However, as cashew plants are well adapted semi-arid conditions, they exhibit great performance when under high temperature conditions (FERREIRA-SILVA et al., 2011). This better performance includes the progressive increase in C_i from 25-35 °C due to higher g_s (DE SOUZA *et al., 2005)* and the stimulation in activity of protective enzymes (FERREIRA-SILVA et al., 2011).

Salinity is characteristic condition of climates with low rainfall and high evapotranspiration rates, such as in arid and semi-arid regions (ETIKALA *et al.,* 2021). In addition, soil salinization is considered one of the major process for soil degradation affecting crop productivity (SMANOV *et al.,* 2023). Around 830 million ha of irrigated areas around the world are expected to be affected by salinity and sodicity issues (MINHAS *et al.,* 2020). Hence, in semi-arid and arid regions, where intensive irrigated systems are even more necessary considering low water availability, it is predicted to increase soil secondary salinization due to higher plants transpiration rates than water supply (CHAVES; FLEXAS; PINHEIRO, 2009). As semi-arid regions often suffers from soil salinization, cashew plants are considered well-adapted to higher NaCl concentrations (FERREIRA-SILVA et al., 2011).

How do cashew plants deal with climate-limiting factors in semi-arid under drought conditions?

Global climate change has extensively increased over the years, augmenting drought incidence, and temperature increment, which adversely affect the morphological, developmental, cellular, and molecular processes in plants (CHAUDHRY; SIDHU, 2022). These constant modifications require a better plants' ability to avoid, be resistant or adapted to changing environmental conditions in the running for survival (CONSIDINE; FOYER, 2021). When plants are exposed to stressful conditions, such as induced by drought, for instance, it could result in the activation conflicting, additive, or completely unrelated molecular responses and different acclimation strategies (RIVERO *et al.,* 2021).

Woody perennial plants are spread in arid and semi-arid regions of the world with mechanisms such as morphological, biochemical, and physiological to deal with limiting factors at these places (AZAM *et al.,* 2012). For cashew plants, our group research has already discussed about some mechanisms which those plants perform under varying environmental conditions, understanding their isolated and/or combined effects, including under drought (VIÉGAS et al., 2001; DE SOUZA et al., 2005; FERREIRA-SILVA et al., 2008, 2010, 2011, 2012; LIMA et al., 2018).

Drought stress

Drought is a meteorological, agricultural and hydrological condition that occurs temporarily or permanently, and causes severe yield loss due to impaired water supply (VICENTE-SERRANO *et al.,* 2020). These phenomena are very relevant for areas with water deprivation, such as the semi-arid regions northeast of Brazil, because the responses occasioned by drought are intrinsic to the natural climatic conditions of these areas for prolonged periods (LIMA *et al.,* 2018).

Plants respond to water availability through physiological, cellular, and molecular events by adjusting of their responses to drought progression (PINHEIRO; CHAVES, 2011). One of these responses is drought tolerance that is the ability of plant in resist to water withdrawal by physiological activities. Thus drought avoidance is the continuity of physiological processes, drought escape the capacity to adjust their life cycle, drought recovery is the ability of plant to restart growth after the exposure to extreme drought stress (ILYAS *et al.,* 2021).

We recently performed the transcriptional profiling of cashew plants (BRS226) under water stress and recovery conditions (CAPELARI *et al.,* 2021). Transcriptomics studies possibilities reveal different acclimation strategies when plants are subjected to stress combinations, inducing unique responses (RIVERO *et al.,* 2021). In the work of Capelari *et al.* (2021), under water deficit conditions, ABA receptor were down regulated, accumulation of genes related to proline biosynthesis and antioxidant protection, and cell wall modification.

Antioxidant response under drought

Drought is also a type of abiotic stress that causes accumulation of ROS such as H_2O_2 and MDA, causing damage to membrane fluidity and chlorophyll structure (AYYAZ *et al.,* 2021). These molecules are accumulated due to an imbalance between ROS production and scavenging (DAS; ROYCHOUDHURY, 2014). Furthermore, the increase activity of antioxidant enzymes such as CAT, SOD, APX, GR and peroxidades (POD), and osmolytes accumulation are some examples adaptive mechanisms under water stress (SUN *et al.,* 2020). Previous studies in cashew plants, demonstrated that part of the oxidative protection under abiotic stress is attributed to SOD and CAT activities, as well as to the use of ASC and GSH (FERREIRA-SILVA et al., 2011, 2012). However, these responses were adjusted according to type of abiotic stress, and if it occurred isolated or simultaneously (FERREIRA-SILVA et al., 2011; LIMA et al., 2018).

ROS are present in plant cells as byproducts of aerobic metabolism and photooxidative processes (DIETZ; MITTLER; NOCTOR, 2016). O_2 is a terminal electron transport with prolific reactive excited states inn ROS which can be formed as hydroxyl radical ($OH^{\cdot}$), superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroperoxyl radical ($HO^{\cdot}_2$), alkoxy radical ($RO^{-\cdot}$), peroxy radical ($ROO\cdot$), singlet oxygen ($^1O_2$) and excited carbonyl (RO^*) (KAPOOR *et al.,* 2015). These molecules also plays import roles on plants under basal levels in signaling pathways for cellular proliferation, metabolic regulation, differentiation and development, signal transduction, for example (MITTLER, 2017).

However, under adverse conditions, such as water and salt stress, and high temperatures, their production can be significantly increased (CONSIDINE; FOYER, 2021). When ROS turns from basal to increased levels, their concentration becomes cytotoxic for plants, and plant cells as chloroplasts, mitochondria, and peroxisomes are the primary sites for ROS formation as a toxic byproduct (MITTLER, 2017). Oxidative protection in plant cells is carried out by a complex defense system, which involves enzymatic and non-enzymatic mechanisms (HASANUZZAMAN *et al.,* 2020).

The non-enzymatic system is mainly composed of hydrophilic components, such as ascorbic acid (ASC) and glutathione (GSH). Ascorbate (AsA) is generated on the inner mitochondrial membrane and transported to different cellular components such as the apoplast playing the main role as scavenger for deleterious ROS with cells (KAPOOR *et al.,* 2015). Gluthathione (GSH) is major found in chloroplasts, but also in the cytosol, endoplasmic reticulum, vacuoles, mitochon dria, peroxisomes, and the apoplast (HASANUZZAMAN *et al.,* 2017). The enzymatic protection is composed of a complex system of enzymes, present in several organelles and acting in a coordinated manner in oxidative protection.

The main oxidative enzymes are superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX), glutathione reductases (GR), monodehydroascorbate reductases (MDHAR) and dehydroascorbate reductases (DHAR), Gluthathione Peroxidase (GPX), Glutathione S-transferase (GST), Thioredoxin reductase (TRX), and Peroxiredoxin (PRX) (HASANUZZAMAN *et al.,* 2020). Together, non-enzymatic and enzymatic antioxidant systems linked to other compounds, such as amino acids and sugars, pigments are powerful systems balancing ROS as oxidant or signaling molecules for acclimation and cell death in plants (NOCTOR; REICHHELD; FOYER, 2018).

High temperatures and drought stress

Heat stress by high temperature occurs when the temperature rises above a threshold and for a period of time sufficient to cause irreversible damage to plant development (WAHID *et al.,* 2007). The definition and damage for heat stress on plant tissues is based in the period and temperature levels during the stress (JAGADISH; WAY; SHARKEY, 2021). Nevertheless, it is important to clarify that not all temperature increases are stressful for plants, instead heat affects plants in a huge range from mild, moderate to severe levels (KAN *et al.,* 2023).

Very high temperatures induce severe heat stress characterized by cell injury, death, and organization collapse in a short period, whereas moderate high temperatures also injure and promote cell death but when plants are under mentioned conditions after longer periods (WAHID *et al.,* 2007). In addition, moderate heat temperature affects plant thermogenesis triggering transition from vegetative to reproductive phases, induce hypocotyl elongation, architectural and morphological modifications (CASAL; BALASUBRAMANIAN, 2019). Another definition for heat stress is heat shock which consist in the exposition to heat treatments within minutes, hours generating molecular and biochemical modifications (JAGADISH; WAY; SHARKEY, 2021).

The heat stress affects plants at different levels (plasmalemma, cytoplasm, nucleus) beginning with changes in plasma membrane increasing the fluidity of lipid bilayer with the increase in calcium efflux, protein denaturation, and ROS generation

(WAHID *et al.,* 2007). The ROS production and lipid peroxidation are both generated in in thylakoid membrane at chloroplast, which can causes PSII inactivation under heat stress (YAMAMOTO, 2016). However, the ROS increase under heat stress can favor the increment in antioxidant activity. Recent works have shown that plants under high temperatures activate enzymatic and non-enzymatic mechanisms for ROS scavenging, as a type signaling response (SILVA et al., 2010; FERREIRA-SILVA et al., 2011; MENDES et al., 2024).

Signaling responses to heat stress are associated with heat tolerance, and they occur by distinct mechanisms such as ion transporters, osmoprotectants, radical scavengers, the proteins abundance and factors involved in signaling cascades, and transcriptional control are some of them (WAHID *et al.,* 2007). To identify the stress levels for plants under high temperature, for instance, it is important to identify different their homeostasis states considering a spatio-temporal perspective (SILVEIRA; SOUSA, 2024).There are some elements in high temperature tolerance as mentioned, the activation of antioxidant defense mechanisms, membrane lipid saturation, increase in gene expression especially for heat shock protein (HSP) genes (*Hsps*) (WAHID *et al.,* 2007).

The negative impacts of high temperature on plant growth can be rapidly observed in short periods, in extended periods, this abiotic stress frequently occurs simultaneously with other stresses, such as drought due to higher evaporation rates augment (MORALES-MARTINEZ *et al.,* 2020). The simultaneously occurrence of heat stress and drought are commonly observed in tropical and sub-tropical environments, inducing several physiological responses such as nutrient cycling, uptake, and photosynthesis (FAHAD *et al.,* 2017). From a meta-analysis study analyzing crop responses to the combination of drought and heat stress COHEN *et al.* (2021) revealed that harvest index, the reduction in the life cycle, altered seed number, size, and composition are the most impacted features in plants.

Nevertheless, these responses also depend on species, genotype, and cultivar. A study involving three tomato cultivars subjected to combined heat and water deficit stress displayed distinct sensitivities, with a predominant effect of drought over heat stress (ZHOU *et al.,* 2017). In addition, GRIGOROVA *et al.* (2011) work showed that the translation of heat shock proteins (HSPs) were significantly modified according to water stress and heat shock treatments in two resistant wheat cultivars. Similar to this response, cashew plants exposed to water deficit were up regulated in the gene expression of heat shock/chaperone genes (CAPELARI *et al.,* 2021). The combination of heat stress with drought is mostly considered as one of most severe factors to cause damage (GRIGOROVA *et al.,* 2011). Nevertheless, when applied with other factors such as salinity, high temperature positively stimulated oxidative protection to salt-stressed plants (FERREIRA-SILVA et al., 2011).

Salt and drought stress

Salt stress affects plant homeostasis and important aspects of plant metabolism, impacting plant growth and yield by photosynthesis, protein synthesis, energy and lipid metabolism (YANG *et al.,* 2020b). Salinity stress can be divided in osmotic stress by the disruption of plant and soil water relation by decrease in water potential and plant turgor increasing osmotic stress and ionic stress by the large Na^+ and Cl^- accumulation, inhibiting K^+ and Ca_2^+ (ARIF *et al.,* 2020).

Salt tolerance, on the other hand, can be divided in three types which can be osmotic (a rapid response) that decreases stomatal conductance with fast long distance signaling mechanisms; in the root system the ionic tolerance is activated by Na^+ influx; and tissue tolerance is the avoidance to toxicity occasioned by ion translocation through their compartmentalization in vacuoles (ISAYENKOV; MAATHUIS, 2019).

In the work of MENDES et al., (2024) salt stressed rice plants subjected to heat shock exposed a series of negative effects of the combination treatments as Na+ accumulation in leaves, osmotic and ionic alterations, which disturbance photosynthetic response by impairment in CO_2 assimilation and decrease in water use efficiency. Additionally, these plants also favored antioxidant responses, which were not enough to avoid increase in membrane damage and decrease in relative water content in leaves (MENDES *et al.,* 2024). However, similar salt and heat combined treatments induced distinct responses in cashew plants. Instead, high temperatures efficiently activate antioxidant protection in cashew plants by an increase in CAT and SOD activity, maintaining the membrane damage integrity(FERREIRA-SILVA et al., 2011).-

In photosynthesis, plants gown under salinity may alter leaf anatomy by changes cell arrangement; chloroplast structure in number, size, lamellar organization; photosynthetic pigments and efficiency (ZAHRA *et al.,* 2022). Under stress conditions, CO_2 fixation might be limited resulting in carbon reduction during the Calvin Cycle and with increase in the NADPH/$NADP^+$ ratio in the chloroplast stroma (FERREIRA-SILVA *et al., 2012)*. As a result, the decrease in $NADP^+$, the main electron acceptor in the electron transport chain, interrupts the electron flux from PSII to PSI which may induce damage to the photosynthetic apparatus (HE *et al.,* 2021). This damage area usually associated to the formation of reactive oxygen species (ROS) that is considerate oxidative stress, occasioned by salinity stress with production of superoxide, hydrogen peroxide and singlet oxygen are produced in chloroplast (MENDES *et al.,* 2024).

However, it's important to highlight that the ROS increment has also positive roles, as these molecules are is essential for redox sensing and signaling for plants (FOYER, 2018). Initially, there a salt stress sensing by proteins and specific molecules responsible to initiate a signaling cascade by calcium under high salinity exposure removing Na^+ excess from the root tissue (FU; YANG, 2023). There are a huge list of transporters which play important role in Na+ removal, such as the SALT OVERLY SENSITIVE (SOS), thus for K+ transporters such as K+ TRANSPORT 1 (AKT1) and HIGH-AFFINITY K+ TRANSPORTER 1 (HKT1) (FU; YANG, 2023).

Photosynthesis and limited process

Plants obtain most of the energy required to survive from CO_2, water and dissolved inorganic ions (minerals). Nevertheless, the energy uptake also requires cost, because, at the same time, plants use energy from a light source to convert CO_2 in metabolites and mineral acquisition (OTORI *et al.,* 2017). Photosynthesis has two main process sustained by the presence of pigments such as chlorophyll and light at photosynthetic apparatus and the generating of ATP and reducing power for CO_2 fixation (MARTIN; BRYANT; BEATTY, 2018).

In the first process, occurs photochemical reactions, which photosynthetic organisms turn the energy obtained from a photon break in new chemical bonds against the chemical equilibrium (HOHMANN-MARRIOTT; BLANKENSHIP, 2011). This consists in the sunlight energy conversion in thylakoids membrane, leading a linear electron transport from PSII to PSI, including the with chemical reduction of $NADP^+$ to NADPH (CROCE *et al.,* 2014). The second process has a crucial role in the balance of CO_2 levels in the atmosphere. In the Calvin–Benson cycle, a ribulose 1,5-bisphosphate (RuBP) molecule carboxylates at the CO_2 acceptor side which is catalysed by Rubisco yielding two mol of the 3-phosphoglycerate (3-PGA) for every mol RuBP carboxylated (YIN *et al.,* 2021).

Considering the biochemical aspects of photosynthesis, the CO_2 assimilation rates can be limited. The kinetic aspects of RuBP regeneration are affected at various levels through electron transport (reduced taxes for electron transport), ATP (insufficient ATP impaired RuBP regeneration), and enzyme (low activity and lack of substrates) levels (FARQUHAR; CAEMMERER; BERRY, 1980; SHARKEY *et al.,* 2007; YIN *et al., 2021)*. These limitations often occur under interference of environmental conditions such as low or high light, high temperatures, and water stress for instance (Sharkey *et al.,* 2007; Yin *et al.*, 2021).

Photosynthesis is one of the most affected physiological processes by drought reducing leaf expansion, the efficiency of photosynthetic machinery, and senescence (FAHAD *et al.,* 2017). These responses are usually a consequence of decreased CO_2 availability and increase in CO_2 diffusion resistance from the atmosphere to the leaves or from the sub-stomatal cavity to carboxylation sites (FLEXAS *et al.,* 2008). Moreover, lower photosynthetic rates under drought stress may occur due to changes in biochemical and/or photochemical activity (ANDRADE *et al.,* 2016). Rubisco concentration and activity can be affected under severe drought stress, which can be explained by a decrease in the de-activation of Rubisco sites when the chloroplast CO_2 concentration (Cc) is low (PINHEIRO; CHAVES, 2011).

In addition, photosynthetic limitation is also affected by CO_2 diffusion. According to Chaves *et al.* (2009), diffusional limitations under abiotic stress such as water and salinity stress are large percentage of total photosynthetic limitation, and sometimes, it has more impact than photo-biochemical limitations (SILVA *et al.,* 2010). In higher plants, for instance, mesophyll (g_m) and g_s accounts for approximately 70% of photosynthetic limitations in angiosperms (GAGO *et al.,* 2020). This response has closure relation with stomatal closure, which is one of the early physiological events under water deficit, reducing CO_2 uptake (FLEXAS *et al.,* 2004).

Stomatal regulation under abiotic stress

Stomata are key structures in the regulatory role for CO_2 uptake and water evaporation (CLARK *et al.,* 2022). These small pores are located at leaf and stems surfaces bouldering by kidney- or dumbbell- shaped guard cells, controlling CO_2 and water vapor exchanges (HETHERINGTON; WOODWARD, 2003; SANTELIA; LAWSON, 2016). The stomatal aperture is driven by guard cell metabolism, which is modified according to their increased volume due to solute accumulation in their vacuoles (DALOSO *et al.,* 2017).

The stomatal turgor is modified by inorganic ions such as potassium (K^+) and chloride (Cl^-) taken from apoplast, whereas organic solutes such as malate and sucrose (Suc) are derived from starch degradation and/or CO_2 fixation in the guard cell chloroplast (SANTELIA; LAWSON, 2016). The K^+ via antiporters channels is the most characterized accumulation process for osmolyte in guard cells (DALOSO *et al.,* 2017). Additionally, recent studies has describing the roles for sucrose and malate coordinating mesophyll and stomatal behavior via the apoplast (Lima *et al.,* 2018; Medeiros *et al.,* 2018; Lima *et al.,* 2019; Freire *et al.,* 2021).

Stomatal are morphologically distinct from others epidermal cells because they have modified metabolic pathways with complex signal transduction networks, and very effective ion transport activity (SANTELIA; LAWSON, 2016). These cells respond to endogenous and exogenous signals such as ROS and phytohormones, air relative humidity, CO_2 concentration and light intensity (Lima *et al.,* 2019). ROS is one of the most important player in plant signalling (MITTLER, 2017). ROS generated in guard cell can have mitochondrial or chloroplast origen delivering funtions such as under drought stress by ABA for stomatal closure with H_2O_2, NADPH oxidases production, calcium channels activation for stomatal closure, for instance (SONG; MIAO; SONG, 2014).

Aiming the gas exchange optimization, stomata developed several functional and adaptative responses to deal with environmental factors (DRIESEN *et al.,* 2020). There are distinct mechanisms underlying stomatal opening to light including the light-spectrum as blue-light acti-vates H^+-ATPase in the plasma membrane with apoplast acidification and K+ uptake, while red-light descrases C_i concentration *(SHIMAZAKI et al.,* 2007). These distinct wave legnths are detected by photorecptors such as criptochromes an phototropins (YANG *et al.,* 2020a).

In a direct response, when CO_2 internal concentrations increases, the stomatal closure is activeted through the despolarization of guard cells plasm membrane, and activation of K+ channels, whereas low *Ci* concentrations triggers (DRIESEN *et al.,* 2020). Futhermore, SLOW ANION CHANNEL 1 (SLAC1) and SLAC1 HOMOLOGUE 3 (SLAH3) are other class of channels with delivery function of anion release to guard cells (Lima *et al.* 2019). The sensibility to high CO_2 concentration additionally alters carbonic anhydrase (CA) enzymes, due to its catalytic activity in the conversion into HCO_3^- and H^+ in guard cells, and the expression of *HIC (HIGH CARBON DIOXIDE)* genes as negative regulator to stomatal development (DRIESEN *et al.,* 2020).

Climate has important effects on stomatal conductance, considering the influence of environmental factors such as temperature, light, CO_2, drought, and vapor pressure deficit (VPD), in the last years, a relevant number of works has studied their impact and correlation with stomatal traits (MOTT, 2009; MAREK *et al.,* 2022; LIU *et al.,* 2023). The evaporation process in plants requires a water vapor gradient in the local site within the stomatal pore, creating a humidity gradient to support vapor flux inside this complex (WONG *et al.,* 2022). Plants remove water from atmosphere through VPD, where variations in this measurement alters guard-cells turgor with stomatal closure and consequently less gas diffusion (INOUE *et al.,* 2021).

When drought occurs, the stomatal conductance might be reduced in response to lower leaf water potential, avoiding water loss by transpiration (FAHAD *et al.,* 2017). High transpiration rates with low levels of water influx into the stem culminate in stomata closure to prevent hydraulic failure, a response observed under high VPD (INOUE *et al.,* 2021), impacting a additionally stomatal density (TULVA; KOOLMEISTER; HÕRAK, 2024). Plants with efficient sensitivity to VPD variations can maximize their transpiration rate efficiency by stomatal conductance (SOUZA *et al.,* 2004).

The stomatal closure in plants under hydric privation, typically precedes detectable changes in leaf water potential and/or leaf water content, making stomatal conductance (g_s) a highly representative and integrative parameter for assessing the overall physiological response to water stress. (MEDRANO *et al.,* 2002).Similar response was observed in the work of CAPELARI *et al.* (2021) which demonstrated how cashew plants subjected to 21 days of water withdrawal maintained stable leaf osmotic potential and relative water content while experiencing a severe decline in stomatal conductance and CO_2 fixation, both nearing zero.

These factors are strongly variable according to climate changes, modifying *A* and g_s ratios for WUE (EYLAND *et al.,* 2021). The WUE has been a key target for crop improvement, because it gives an important determination about the amount of carbon gain relative to water use, which is strongly affected by transpiration and VPD (LEAKEY *et al.,* 2019). The control of stomata aperture allows plant to maintain photosynthetic activity under lower transpiration rates and conductance, increasing WUE (PETERS *et al., 2018)*. Thus, stomata optimize WUE protecting the water transport tissues from embolism in the vascular system, and acting as a protective mechanism for rapid closure response under high transpiration rates or soil dehydration (BRODRIBB; SUSSMILCH; MCADAM, 2020). Under drought stress, the ratio of leaf-internal and atmospheric CO_2 (Ci/Ca) rates and stomatal conductance are expected to decrease, reducing photosynthetic activity and increasing the WUE by stomatal aperture control (PETERS *et al.,* 2018). The positive effects of an increase in WUE under drought stress for crop yield by sustainable carbon gain with an equivalent amount of water uptake depends on whole-plant response and can vary according to the environment, water deficit magnitude, genotype (LEAKEY *et al.,* 2019).

Because stomatal behavior is influenced by changing environmental conditions, stomatal kinetics have been used to predict how plants adjust CO_2 uptake and water loss, incrementing intrinsic water use efficiency ($_i$WUE) (LAWSON; MATTHEWS, 2020). For this reason, in the past years has been emerged numerous works for light and CO_2 stomatal kinetics optimizing maximum fitness for these models and their description analyzing *A* and g_s (MRAD *et al.,* 2019). The synchrony between *A* and g_s may be disrupted by slow stomatal responses, potentially leading to a reduction WUE (LAWSON; VIALET-CHABRAND, 2019). Under a field environment, light intensity is one of the most unstable environmental conditions, where *A*, g_s, and WUE responses to step changes have been used to understand stomatal dynamics under these conditions (EYLAND *et al.,* 2021).

A great number of studies have investigated the dynamics of stomatal response and photosynthesis to light fluctuation under constant condition in steady state that differently from field conditions, gives a higher correlation response (VIALET-CHABRAND *et al.,* 2017). Mostly plants specially angiosperms, when exposed to high CO_2 concentration increase it uptake by *A* reducing g_s (AINSWORTH; ROGERS, 2007). However, LIMA *et al.,* (2019) showed that stomatal movement varied among species, which could be associated with evolutive response. Thus, stomatal structure, dynamics, and biochemical properties play a crucial role in enhancing photosynthesis and water use efficiency, remaining largely untapped opportunities for modification to optimize both processes and their balance in agriculture.

Concluding remarks

As a well-adapted evergreen plant species to the tropical semi-arid regions of Brazil, and as its importance as income for many other countries, cashew plants (*Anacardium occidentale* L.) fit an important crop model to be an object of study. Despite many works that have already analyzed some physiological, biochemical, and molecular adjustments of cashew plants under a range of environmental conditions, new studies are still required. In this work, we performed a series of experiments to get insights into stomatal, photosynthetic, and water deficit reponses in cashew plants. We precisely chose these topics, aiming to obtain a deep understanding of them, and of course, raise new questions.

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4 DIFFERENTIAL INDUCTION KINETICS OF STOMATAL CONDUCTANCE AND CO_2 ASSIMILATION IN RESPONSE TO WATER DEFICIT AND REHYDRATION IN CASHEW LEAVES

Manuscript in submission

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Abstract - Stomata are a key balancer for photosynthesis and transpiration rates, affecting CO_2 uptake and water vapor loss. Under limiting environmental conditions their response may differ to optimize these exchanges. A great number of researches have been characterizing how stomata limit CO_2 assimilation (A). However, additional work is still required for the temporal understanding of stomatal control and water use efficiency (WUE) under water deficit. Here we used an important drought-tolerant crop, cashew plants (*Anacardium occidentale* L.) to investigate how this species deals with water deficit and which role has stomata in this tolerance. Our results showed that stomatal conductance (g_s) strongly limits A during step change light transition under water deficit in cashew plants. In addition, we demonstrated that mild water deficit positively stimulated g_s incrementing photosynthetic response. Under the light stimulus, cashew-recovered plants reversed A and g_s decrease with a change in stomatal closure response, resulting in better WUE. Furthermore, we found that cashew plants, changed completely the stomatal behavior under light kinetics, but were less sensitive to step change CO_2 transition, which is an unusual response for angiosperms plants. These results imply the relevance of stomatal conductance as mechanistic responses to deal with water deficit conditions in cashew plants.

Keywords: drought**;** g_s; photosynthesis; recovery; water use efficiency; *Anacardium occidentale* L.

INTRODUCTION

From an evolutionary concept, stomata are key structures that work as valves for plant-land adaptation due to their regulatory role for CO_2 uptake and water loss through transpiration (CLARK *et al.,* 2022). Together with the development of other specialized structures such as roots, vascular tissues, and cuticles, the presence of guard cells, which compose the stomata, induced in early terrestrial photosynthetic organisms, the development of a system for soil water uptake, light absorption, and CO_2 assimilation (BERRY; BEERLING; FRANKS, 2010). Guard cells are specialized cells in the stomatal complex; that function as pore regulators in the opening and closing processes (DALOSO *et al.,* 2017).

This complex is a key balancer for photosynthesis and transpiration rates, affecting CO_2 uptake and water vapor loss (CLARK *et al.,* 2022). Since the Phanerozoic era, density and morphological changes have shown a high correlation between climate change and the stomatal evolutive process, where environment signals are one of the agents controlling its aperture and development (HETHERINGTON; WOODWARD, 2003). Physiological model approaches for stomatal apertures define shifts in guard cell volumes as a result of water movements following the osmotic gradient within and around these cells (BUCKLEY; MOTT, 2013; BUCKLEY, 2017). The incidence of environmental perturbations can alter a plant's water potential, and transpiration rate inducing stomatal partial closure (BUCKLEY, 2019).

As a meteorological, agricultural, and hydrological environmental condition, drought causes severe losses due to inefficient water supply (VICENTE-SERRANO *et al., 2020)*. Plants have developed several drought resistance mechanisms some of which can be classified as drought avoidance (DA), drought tolerance (DT), drought escape (DE), and drought recovery (FANG; XIONG, 2015). Under water stress, plants with DT mechanisms may precisely adjust both stomatal and mesophyll conductance (PINHEIRO; CHAVES, 2011). The amount of CO_2 and H_2O flux is determined by photosynthesis (*A*) and transpiration (E) rates (DALOSO *et al.,* 2017). Stomatal aperture regulation makes plants able to maintain photosynthetic activity with lower transpiration rates and stomatal conductance increasing the water use efficiency (WUE), which is one of the parameters most affected by drought (PETERS *et al.,* 2018).

The WUE can be defined as the amount of accumulated biomass per unit of water used or transpired by the plant (FREIRE *et al.,* 2021). Once stomata orchestrate the balance between CO_2 uptake and *E*, it is known the existence of a strong correlation between *A* and g_s (MCAUSLAND *et al.,* 2016). Although the intrinsic water use efficiency (iWUE) between photosynthesis and stomatal conductance is normally a desired characteristic for crop improvement, it represents a partial cost for photosynthesis as the *A* is limited by g_s (EYLAND *et al.,* 2021). Environmental factors such as water availability, temperature, light, and CO_2 may interfere with stomatal regulation (MOTT, 2009; MAREK *et al.,* 2022; LIU *et al.,* 2023) and consequently in WUE. In normal environmental conditions, their intensity and presence are strongly variable according to climate changes, modifying *A* and g_s ratios for WUE (EYLAND *et al.,* 2021). For this reason, in the past years has been emerged numerous models for stomatal kinetics considering the impact of environmental cues optimizing maximum fitness for these models and their description (MRAD *et al.,* 2019).

The control for stomata opening and closure is sensitive to light and CO_2, thence recent studies have focused on exploring how plants respond to those stimuli across different plant species and plant metabolism (LIMA et al., 2019; CÂNDIDO-SOBRINHO *et al.,* 2021; FREIRE *et al.,* 2021), and WUE (MCAUSLAND *et al.,* 2016; EYLAND *et al.,* 2021). An example of this was observed in the work of MEINZER et al., (2017) with isohydric and anisohydric plant species, which have distinct leaf and soil water potential daily minimum control, responded differently for *A* and g_s under stomatal kinetics and photosynthetic gas exchange.

The work of Capelari *et al.* (2021) showed that cashews exposed to gradual water deficit performed with isohydric responses by stomatal closure in the early stages of water deficit. The stomatal regulation according to isohydric and anisohydric responses occurs as a survival function under a limited water supply. While isohydric plants display a rapid response to soil dehydration with lower CO_2 assimilation rates and growth, plants with anisohydric responses take water availability from the soil up to lower potentials, reaching higher photosynthetic rates and adjustments of stomatal conductance and leaf transpiration (CHAVES *et al.,* 2016). These statements raise a big question about how plants deal with water availability under unfavorable environmental conditions by photosynthetic and stomatal adjustment.

Cashew (*Annacardium occidentale* L.) is a tropical crop plant that grows extensively in the northwest of Brazil, which for the most part is composed of semiarid regions (FERREIRA-SILVA *et al.,* 2010). It's believed that cashew plants possess evergreen physiological characteristics due to their ability to deal with extreme environmental conditions (Lima et al. 2018). Some studies have already suggested that cashew plants display low photosynthetic rates (SILVEIRA *et al.,* 2003; DE SOUZA *et al.,* 2005) with a balance in light capture and photosynthetic electron consumption by metabolic processes under drought and high light (LIMA *et al.,* 2018). In this study, we tested the hypothesis that cashew plants have a low sensitivity to water reestablishment for the A and g_s restoration due to low plasticity under water deficit. To understand this question, we used a cashew genotype which has low plasticity and has low response for photosynthesis improvement under irrigated systems

In our work, we first demonstrated and characterized stomatal responses to light and CO_2 in the cashew genotype (BRS226) under water deficit and rehydration events. We observed that even when plants did not display prominent physiological stress indicators by water deficit, stomatal conductance is strongly limited after light and CO_2 transitions. However, its slow recovery after closure occurs in a progressive and efficient process. By this finding about stomatal responses to light and CO_2 of an important crop plant well adapted to multiple environmental cues, we believe that we will extend the understanding of the photosynthetic mechanism of drought tolerance in plants adapted to semiarid conditions.

MATERIAL AND METHODS

Plant growth and experimental conditions

Anacardium occidentale L. grafted seedlings, BRS226 genotype, were provided by Embrapa, Brazil. Plants were transplanted to 10L plastic pots filled with sand: vermiculite: vegetal mix substrate (2:1:1) (v/v) and nutritional supplemented with ½ the Hoagland-Arnon nutrient solution (HOAGLAND; ARNON, 1950), The mix substrate was irrigated at field capacity with distilled water and after 1 week of cultivation, the nutrient solution was changed to full strength and applied weekly. Plants were grown under similar conditions at a greenhouse located at 3°44′44.2″S

38°34′ 29.2″W. During the experiment, the environmental parameters were monitored registering day/ night variations in temperature (25–32° C), relative humidity (73–89%), and a photoperiod of 12 h. Daily light monitoring range varied from 100 to 1100 µmol m^{-2} s^{-1} PPFD. This process was performed twice in separate periods to establish the experiment I and II.

Experiment I: growth establishment

Cashew plants with up to ± ten expanded leaves (45 days old) were divided into three groups according to the hydric regime supplied: (0WD) control, non-water deficit; (21WD) twenty one days of water deficit; (REC) five days of recovery with water rehydration supply. The time for water deficit was stabilized according to previous experiments for the optimal timing of severe reversible stress. This experiment was designed following the methodology of CAPELARI *et al.,* (2021). From this experiment we performed net photosynthesis curves in response to changes in photosynthetically active photon flux (*A/PPFD*) in fully expanded leaves.

Experiment II: growth establishment

Cashew plants with up to ± forty expanded leaves (5 months old after transplant) were maintained in these growth conditions for acclimation and to reach a development stage closer to that found in the natural environment. The plants were divided into six groups according to the hydric regime supplied: (0WD) control, non-water deficit; (4WD) four days of water deficit; (8WD) eight days water deficit; (12WD) twelve days of water deficit; (2REC) two days of recovery with water rehydration supply; (4REC) four days of recovery with water rehydration supply. We chose the most contrasting hydric regimes for the main results: 0WD, 12WD, and 4REC. From this experiment, we performed light and CO_2 kinetic curves.

Physiological stress indicators: electrolyte leakage (EL), relative water RWC) content, total plant and soil evapotranspiration (ET_0), and leaf osmotic potential (Ψo)

Relative water content (RWC) was measured according to leaf discs weighing (fresh, fully saturated, and dry), and electrolyte leakage (EL) (SHANAHAN et al., 1990). Ten discs of cashew leaves were added to 10 ml of distilled water and mixed overnight. After 24h, electrical conductivity (C1) was determined, the extract was boiled for 1h and the new electrical conductivity (C2) was determined. The EL was expressed according to the following equation % EL = (C1 / C2) * 100 as previously described (CAVALCANTI et al., 2004).

Gas exchange parameters

The steady-state for net CO_2 assimilation (*A*), stomatal conductance (g_s), transpiration rate (*E*), intercellular CO_2 partial pressure (Ci), ambient CO_2 concentration (Ca) were measured in fully expanded leaves from cashew plants with a portable infrared gas analyzer system equipped with an LED source and a leaf chamber (IRGA LI-6400XT, LI-COR, Lincoln, USA). The internal parameters inside the IRGA chamber during gas exchange measurements were 1000 μmolm^{-2} s^{-1} PPFD, 1.0 ± 0.2 kPa VPD, 400 ppm CO_2 and 28 °C. Blue light amount was set to 10% of the PPFD to maximize the stomatal aperture (FLEXAS *et al.,* 2008).

Net photosynthesis curves in response to changes in photosynthetically active photon flux (*A/PPFD*) were determined in fully expanded leaves from cashew plants varying the PPFD increasing and reducing of light reference from 0 to 2000 μmolm^{-2} s^{-1} PPFD under 400 ppm. The data were analyzed using the *A/*PPFD to estimate photosynthetic parameters (*A/max*, *1/Φ,* LCP) from curve Fitting 10.0 utility available at http://landflux.org/Tools.php. The LSP was calculated according to MARTINS *et al.,* (2013) adjustment from curve Fitting 10.0 utility available at http://landflux.org/Tools.php. We also estimated from *A/*PPFD the Rd and *Jmax* (SHARKEY *et al.,* 2007).

Stomatal and photosynthetic kinetics responses during light transitions

Gas exchange during dark-to-light (0–1000 μmol m^{-2} s^{-1}) and light-to-dark kinetics kinetics (1000–0 μmol m^{-2} s^{-1}) were performed using a Walz GFS-3000 gas exchange system with 10 cm^2 (Heinz Walz GmbH, Effeltrich, Germany). During the experiment setup, previous tests confirmed the time to reach stomatal conductance steady state on cashew plants (40 min). Cashew leaves were conditioned inside the cuvette for 20 minutes under dark conditions (0 μmol m^{-2} s^{-1}), 40 m under light (1000 μmol m^{-2} s^{-1}), plus 20 m in the dark (0 μmol m^{-2} s^{-1}. The gas exchange parameters were recorded every 16 s for 1200s (dark), 2400s (light), and 1200s (dark) under 400 µmol CO^2 mol^{-1} and 30° C of block temperature. Each point on the curve represents the mean of 10 measurements taken throughout the kinetic assay (FREIRE *et al.,* 2021).

The half-time steady state g_s (t50 %) were estimated by the time recorded during g_s kinetics to achieve 50 % of the steady state (LIMA *et al.,* 2019). The calculation of maximum slope of g_s response (*Slmax*) was obtained according to McAusland *et al.* (2016) by the *dgs/dt* when the acceleration plateau is reached. Thus, a non-linear regression equations was applied to activity the rate of change for stomatal opening and closure, and the data was a linear plateau model to estimate *Slmax* in stomatal opening and closure (MCAUSLAND *et al.,* 2016). The *gmax* was obtained from the steady state from dark and light conditions.

Stomatal and photosynthetic kinetics responses over CO_2 concentrations changes

Gas exchange during changes CO_2 concentrations (400-800 ppm) were performed using an infrared gas analyser (IRGA) with 2 cm^2 (Li-Cor 6400XT; Li-Cor, Lincoln, NE, USA). Plants passed through acclimation period before the kinetics started. During the experiment setup, previous tests confirmed the time to reach stomatal conductance steady state on cashew plants according to CO_2 concentration (40 min). Then, gas exchange parameters were recorded every 10 s for 2400s (400 ppm), plus 2400s (800 ppm). Each point on the curve represents the mean of 10 measurements taken throughout the kinetic assay (FREIRE *et al.,* 2021). The calculation of maximum slope of g_s response (*Slmax*) was obtained according to McAusland *et al.* (2016) by the *dgs/dt* when the acceleration plateau is reached. Thus, a non-linear regression equations was applied to activity the rate of change for stomatal closure, and the data was a linear plateu model to estimate *Slmax* in stomatal closure (MCAUSLAND *et al.,* 2016).

Determination of plant intrinsic water use efficiency (iWUE)

Stomatal kinetics curves of *A* and g_s were assessed under steady state (1000 µmol photons $m^{-2}s^{-1}$ and 400 ppm) to calculate their rates of obtaining the intrinsic water use efficiency (iWUE) (FREIRE *et al.,* 2021)

Experimental design and statistical analyses

The experiment was completely randomized with six water regime levels and four biological replicates of one plant by pot (6 x 4), twenty-four plants. Data were analyzed by analysis of variance (ANOVA), and the means were compared using Tukey's test ($P < 0.05$) or by Student's t-test at 5% probability ($P < 0.05$) using Genes Software (CRUZ, 2013). The dataset was further analyzed through a multivariate approach by Partial Least Squares Discriminant Analysis (PLS-DA) and a Variable Importance in the Projection (VIP) using MetaboAnalyst platform (CHONG *et al.,* 2018).

RESULTS

Physiological stress indicators in cashew plants subjected to water deficit and recovery

This work evaluated the physiological response levels of cashew plants (BRS226) subjected to water deficit. Firstly, we performed a photosynthetic characterization on *Experiment I* inducing severe water deficit (Fig. S1). Then, based on the responses obtained from *Experiment I*, we performed *Experiment II* inducing a progressive water deficit (WD) (0, 4, and 12 days) and rehydration recovery (REC) (4 days) (Fig. S1). Changes in water availability (0WD, 12WD, and 4REC) did not result in a significant difference in relative water content (RWC) (Fig. 1A). Similarly, as a stress indicator, lipid peroxidation measured by electrolyte leakage did not differ between 0WD, 12WD, or 4REC treatments (Fig.1B). These plants showed visual controversial morphological variations (Fig. S2A, B, and C). The senescence process on cashew plants was modified by water deficit, as the number of expanded leaves (fully developed) was significantly decreased after 12WD (Fig. S2D). Interestingly, cashew plants kept growing even under 12WD treatment as the number of new leaves (unexpanded leaves) from the apical meristem increased significantly compared to well-watered plants (Fig. S2E). Thus, this response was similarly observed in plants after 4 days of rehydration treatment (4REC) (Fig.S2E).

Cashew plants exposed to 12 days of continuous water deficit strongly decreased transpiration rates (*E*) by ±33% compared to well-watered plants (Fig. 2A). Remarkably, *E* reduction was completely reversed after 4 days of rehydration (4REC) (Fig. 2A). Following this response, total plant evapotranspiration (ET_0) rates increased significantly compared to plants under 0WD and 12WD treatments (Fig. 2B). 12 days of water deficit discreetly decreased leaf osmotic potential in cashew plants (Fig. 2C). On the other hand, the rehydration treatment rescued to similar.

How cashew plants respond to water deficit and recovery?

In order to understand how cashew plants differently respond to progressive increment to water deficit, we started the experiment with photosynthetic characterization. Photosynthetic rates and stomatal conductance steady-states monitored in cashew plants among 29 days were strongly modified according to stress level (mild or severe) (CAPELARI *et al.,* 2021) (Fig. S4 A, B). In addition to significant decreases in relative *A* and g_s, these authors demonstrated a relevant delay in recovering both parameters after rehydration (CAPELARI *et al.,* 2021). Therefore, as plants under 5 days of re-hydration treatment did not fully recovered from 21 days of water deficit, additional photosynthetic analyzes are still required.

Gas exchange of light response curves

Kinetics of gas exchange response curves under increasing light intensities and dark-light-dark transitions were performed to understand how cashew plants adjust their photosynthetic responses under water deficit and recovery events. Firstly, Net CO_2 assimilation (*A*) and stomatal conductance (g_s) curves in response to changes in photosynthetically active photon flux density (PPFD) were measured as screening to see how cashew plants respond to light increment under WW (well-watered), WD (21 days of water deficit), and REC (5 days of water rehydration) treatments (Fig. 3A).

Well-watered cashew plants progressively increased their CO_2 assimilation according to light increment (Fig. 3A). However, when plants experienced a long water deficit period (21 days), the increments on *A* according to light were maintained without a progressive increase (Fig 3A), reducing their estimation for maximum photosynthesis levels (*Amax*) (Table S1). In addition, the saturation point (LSP) for these plants was significantly reduced by almost 50%, which negatively impacted the light use efficiency ($1/\phi$) (Table S1). On the other hand, the REC treatment (5 days of re-watering) efficiently recovered more than 50% of the *A*/PPFD levels compared to plants under WD (Fig. 3C). Thus, REC plants at 500 μmol m^{-2} s^{-1} reached more than 70% recovery compared to WD plants at the same light curve point without significant differences from well-watered plants (Fig 3C). Although, recovered plants partially restore their CO_2 assimilation rates, their previous under water withdrawal significantly decreases their *Is* and *Amax* (Table S1). Therefore, the maximum rate of photosynthetic electron transport (*Jmax*) was significantly decreased in these plants (Table S1).

Similar to the *A/PPFD*, g_s*/PPFD* curve showed that after water deficit periods, plants consistently modified their stomatal conductance (g_s) (Fig. 3B). The g_s levels were significantly decreased in WD (21 days of water deficit) and REC (5 days of recovery) treatments independent of light intensities (Fig. 3B). Thus, stomatal conductance was more affected by water deficit treatments as the g_s relative recovery showed lower recovery ability on WD and REC treatments compared to *A* recovery responses (Fig. 3C, D).

To properly understand cashew plants photosynthetic adjustment under WD and REC treatments, our next step was to analyze the net CO_2 assimilation (*A*) and stomatal conductance (g_s) kinetic under light transitions (Fig. 4) (Table S1). For this analysis, we performed the *Experiment II* choosing a different period for treatments (Fig S1) as *A* and g_s of cashew plants did not respond to the light stimulus (1000 μmol $m^{-2}s^{-1}$) after 12 days of water deficit (12WD) during kinetics (Fig. 4A, B).

The raise in *A* during dark to light transitions (0-1000 μmol $m^{-2}s^{-1}$) in cashew plants was differently affected by water availability (Fig. 4A.1, A.2). Responses for *A* were remarkably affected under light by 12WD treatment, inducing significant differences compared to well-watered plants (Fig. 4 A.2). Plants under 12 WD slowly increased their photosynthetic levels under light (20-60 min), whereas a rapid increment in photosynthesis was noticed on 0WD, 4WD, and 4REC plants (Fig. 4A.1). After a light-dark imposition, the A levels instantaneously decreased by almost zero among all treatments as a light response (Fig. 4B1). In stomatal kinetic responses under light transitions, we noticed a very distinct response from (Fig. 4B.1). Well-watered, 4WD, and 4REC strongly incremented their stomatal opening from 0-1000 μmol $m^{-2}s^{-1}$ (Fig. 4B). This was additionally observed by the maximum slope (*Slmax*) of stomatal conductance (Fig. 5A). The g_s of 12WD plants were almost decreased to zero along all kinetic, showing unaltered opening or closing responses during light transitions (Fig. 4B.1). Therefore, the *Slmax* of plants under 12WD was approximately unchanged during opening or closing kinetic periods (Fig. 5 A, B). The g_s drastic decrease was related to the impact in number of days of water withdrawal (12WD) as plants under 0WD, 4WD and 4REC did not differ significantly (Fig. 4B.1). Thus, there was no statics difference between 0WD and 8WD treatments in *A* and g_s individual kinetics as well for *slmax* opening (Fig. 5A). In contrast, slmax closing of 8WD plants was statistically reduced compared to 0WD, 4WD, and 4REC treatments (Fig. 5B).

Stomatal conductance characterization parameters such as t50% and gsmax were modified in the presence or absence of light (Fig. 5, B). The half time to reach g_s steady state (t50%) was increased in plants under 4 and 8WD water withdrawal under light (Fig. 6A). This increment represented ±50%, when plants were subjected to 4WD (Fig. 6A), which was followed by the maximum theoretical stomatal conductance (*gmax*) under light (F. 6B). The 12WD treatment negatively impacted the t50% with a severe decrease, similar in *gmax* in the light (Fig 6B). Curiously, 2REC and 4REC treatments increased their t50% to similar levels for well-watered plants (Fig. 6A). In addition, a progressive increment was observed in these treatments for *gmax* under light conditions (Fig. 6B). Nevertheless, in the light absence of well-watered (0WD) and recovered plants (2REC and 4REC) achieved highest values for t50% (Fig.7B). These responses were not visualized in plants after water deficit treatments, once their t50% values were significantly decreased (Fig. 6A).

iWUE and Ci/Ca responses to step-changes in light intensity

The iWUE was modified according to treatments (Fig. 7B). Overall, this response was positively stimulated under dark-to-light transition for all treatments (Fig. 7B). 4REC plants had significant iWUE increments during initial light exposition, even in the presence of a slight decrease according to time (Fig. 7B). As *A* in plants under 12 WD was expressively affected, the beginning of iWUE kinetics, they started very negatively (Fig. 7B). Moreover, plants from this treatment almost did not respond to dark-to-light-to-dark kinetics. Their maximum iWUE was reached only at the last points, after 60 min, whereas for other treatments their maximum responsiveness was at 30 min. Similar to *A*, plants post-illuminated instantaneously decreased iWUE under dark (Fig. 7B). additionally, the same response was observed for plants under 08WD and 2 REC (Fig. S4B).

By iWUE responses, plants under 4REC treatment positively regulated the internal and external CO_2 concentration (Fig. 7A). In addition, plants under moderate water deficit (4WD) positively regulated Ci/Ca responses, as this parameter was significantly decreased compared to control plants. The water deficit treatment significantly increased Ci/Ca, compared to control (Fig. 7A). Thus the same response was observed in 08 and 02 plants (Fig. S4).

Photosynthetic responses of cashew plants under CO_2 concentration transitions

To understand how the photosynthetic and stomatal responses changes from ambient (400 ppm) to elevated (800 ppm) CO_2 concentrations in atmosphere, we performed an experiment to understand this question in cashew plants under different water regimes (Fig. 8). Similar to dark-to-light and light-to-dark kinetic transitions, 4WD and 4REC showed higher CO_2 rates under 400 ppm. Thus, plants under 12WD treatment reduced *A* and g_s severely (Fig. 8A, B). Photosynthetic rates did not differ among treatments from ambient to high CO_2, remaining mostly in the steady-state during all kinetic (Fig 8A), (Fig. S4A). However, only plants exposed to 12WD treatment retarded their *A* increment during the first 20 minutes of induction (Fig. 8A). In addition, only 12 WD plants exposed slight variations in *A* during induction in both CO_2 concentrations (Fig. 8A).

The cashew stomatal conductance was differently affected during kinetic CO_2 transitions (Fig. 8B). Plants under 0WD, 4WD, 12WD had faster response to increased CO_2 concentration as their *Slmax* were significantly increased (Fig. 9). These plants exposed a rapid decline after CO_2 transition in the interval between 40-50 min during kinetics followed by their steady-states (Fig. 8B). Interestingly, we observed that after recovery treatments of 2 or 4 days, g_s was less sensitive to increased CO_2 concentration as their stomatal remained opened during kinetics (Fig, 8B) and (Fig.S4B). Thus, the lowest *Slmax* under CO_2 transitions was observed on recovery plants (Fig. 9).

iWUE and Ci/Ca responses to CO_2 transitions

Differently from the light transition experiment, the iWUE was less stimulated on CO_2 kinetic transitions (Fig. 8C). This response was affected by the g_s levels as the stomata remained opened or partially opened in most treatments (Fig. 8B). In the light transitions kinetic, cashew plants exposed to 12WD had the worst iWUE (Fig. 7B). However, when during CO_2 transitions, these plants performed better iWUE under ambient and high CO_2 with significant differences compared to 0WD (Fig. 8C). The iWUE of 0WD, 4WD, and 4REC rapidly reached their steady-state with minimal changes during the kinetic routine (Fig. 8C). The decreases in Ci/Ca relation for 12WD confirm the iWUE response with a better CO_2 consumption (Fig. 9). Another interesting response was the positive effect of 4 days of water-withdrawal, decreasing Ci/Ca of these plants Fig. 9).

Multivariate data analysis in cashew plants response to water deficit and recovery treatments

We applied Principal Component Analysis (PCA) multivariate analysis to understand by a systemic view, how variations in water regimes of cashew plants differentiate statistically the treatments. Twelve parameters related to stress and photosynthetic indicators were analyzed to seek their discrimination between treatments. The 2D score plot PCA analysis displayed the distributions on the component 1 (78.3%) was more relevant than component 2 (9.5%) (Fig.10). As it analysis covered more than 80% of total variation, we considered it as a good comprehensive view of this experiment data variability. The groups were clear and well separated according to treatment interference along the axis of PC1 component, suggesting that most physiological and photosynthetic responses were differently modified according to water availability (Fig. 10). The minimal overlaps and regions occupied by ellipses suggested a good confidence interval of 95% for 12WD and 4REC treatments.

DISCUSSION

Understanding the signs of water-deficit resistance in cashew plants

In this study, as expected, the responses observed in cashew plants were of drought resistance. Under water deficit, one of the first symptoms usually visualized in plants susceptible to drought is growth limitation, which is commonly demonstrated by leaf area, and morphological adjustments. Drought-tolerant plants possess types of morph-physiological response mechanisms during water privation such as in leaf structure, and osmolyte adjustment (ILYAS *et al.,* 2021). From our characterization, cashew plants did not modify their hydric status when they were under water deficit without very significant changes in RWC, EL, and leaf osmotic potential (Fig. 1), (Fig. 2C). Probably, these adjustments were more related to drought avoidance with mechanisms for water saver to active higher water use efficiency (WUE) (BODNER; NAKHFOROOSH; KAUL, 2015). The cashew plant's ability to maintain the leaf hydric status even under water deficit has been reported in the literature (MATOS; TEXEIRA JUNIOR; SILVEIRA, 2003; LIMA *et al.,* 2018) which corroborates with this work.

We did observe some morphological changes as the number of mature leaves (fully expanded) significantly decreased under moderate water deficit (12WD). Interestingly, we also noticed that cashew plants did not interrupt their growth, as they invested in the growth of new leaves even during water-withdrawal treatment (Fig. S2). Both of these morphological adjustments might be related to phytohormone crosstalk, as these molecules are recognized in the control of leaf senescence and plant fitness (JAN *et al.,* 2019). Plants under drought conditions normally increase abscisic acid levels (ABA), and as cashew plants were exposed to moderate periods of water deficit, this hormone might affected the leaf senescence processes (MAO *et al*., 2017; JAN *et al*., 2019). Recently, there was found a coordination response under drought stress by GA-ABA crosstalk in rice plants, which SLENDER RICE 1 (SLR1) and Tiller Enhancer (TAD1) protein accumulation and degradation are precisely regulated by these phytohormones, enhancing growth or not (LIAO *et al*., 2023).

Cashew plants under water deficit (12d) strongly decreased their transpiration rates (*E*). Under drought stress, a decrease in transpiration rates and stomata closure is one of the first arrangements for drought tolerance (ILYAS *et al*., 2021). Indeed, we observed that cashew plants' g_s were drastically decreased in the mentioned treatment (Fig. S9). This coordinator response was expected as stomata are major controllers between internal and external gas exchange, as well as the water loss diffusion by *E* (LAWSON; VIALET-CHABRAND, 2019). Normally, decreases in *E* are followed by reduced leaf area, as the cell wall of leaf tissues is less extensible under water deficit (TARDIEU; GRANIER; MULLER, 2011). However, cashew plants under severe water deficit (21d) did not display significant differences in cell expansion by leaf area (Fig S3). Despite this controversial response, this could be explained as a stomata adaptive response. In a grass native plant species, XU; ZHOU (2008) related that leaf stomatal size and density were consistently modified according to water stress levels. In their study, the morphological traits remarkably altered gas exchange lower transpiration rates were not directly correlated to leaf area, but instead with stomata density and stomata size.

Understanding stomatal regulation in cashew plants under water deficit: may they control water deficit resistance?

We wanted to look at how stomatal conductance might be altered under water deficit. For this, we started analyzing a previous work from our group to understand how photosynthesis and stomatal conductance are regulated under water deficit and recovery periods (CAPELARI *et al*., 2021). The net CO_2 assimilation and g_s responses under water deficit (21d) and recovery (5d) relative to well-watered treatment were similarly decreased; however, after water reestablishment *A* increased more efficiently than g_s (Fig. S4). In general, this response is not similar to those observed in other C_3 plants. The work of SOUZA *et al*., (2004), for example, demonstrated that cowpea plants (*Vigna unguiculata*) had all photosynthetic parameters recovered after 3 days of re-watering.

On the other hand, is important to consider the applied experimental strategy and species studied. In *Eucalyptus camaldulensis* plants, the recovery of photosynthetic parameters as A and g_s was dependent on the degree of antecedent water deficit and drought severity, showing a faster recovery response for those plants that already passed through water deficit events (LI *et al*., 2021). Nevertheless, it is important to highlight that although cowpea plants presented full recovery in a short period, they have additionally activated photosynthetic, biochemistry, and photochemistry to return to their initial levels (SOUZA *et al*., 2004). In the work of LI *et al.* (2021), they attributed smaller reductions in *Amax* to biochemical components of photosynthesis rather than gas exchange, as g_s was not differed among drought treatments. Because cashew plants are mostly cultivated in semiarid regions with the constancy incidence of multiple limited conditions, such as water deficit (BEZERRA *et al*., 2007), the early stomatal closure and slow recovery response might be associated adaptive mechanisms (CAPELARI *et al*., 2021).

To understand how photosynthetic adjustment occurs in cashew plants under water deficit, we then analyzed their response to the *A/PPFD* curve. Cashew plants are well-adapted to extreme climatic conditions, including high light, for this reason, we performed an *A/PPFD* with a light saturation point of up to 2000 μmol m^{-2} s^{-1}. Under these conditions, we noticed that the cashew gas exchange response was stimulated in response to light increment, but under water deficit (21d) and recovery (5d), plants maintained a similar response after 500 μmol m^{-2}s^{-1}. This such a controversial response, as in previous work, cashew plants under water deficit had higher A and WUE under high light than under low light conditions (LIMA *et al*., 2018).

Nonetheless, past work from our group has already shown that cashew plants activate non-enzymatic and enzymatic mechanisms as antioxidant responses under salt stress (FERREIRA-SILVA *et al*., 2011). In *Arabidopsis thaliana* plants acclimated to low light or natural growth chamber light (8-80 μmol m^{-2}s^{-1}), close to their light compensation, for instance, did not suffer from oxidative stress after a change to 100- and 10-fold light jump by the activation oxidative regulatory mechanism in most parts from the water-water cycle (OELZE *et al*., 2012). Thus, as cashew plants possess a relevant involvement of water-water cycle and antioxidant enzymes, as well as a powerful photoprotection mechanism at light-harvesting antenna for energy dissipation by non-photochemical quenching (NPQ) (LIMA *et al*., 2018). These could explain why even at moderate light saturation point, high light increment did not affect CO_2 uptake. Under drought stress, diffusive CO_2 limitations are more predicted to occur than biochemical limitations (FLEXAS *et al*., 2004). However, cashew plants under recovery treatment (5d), significantly decreased their *Jmax*, suggesting negative effects on the biochemical phase of photosynthesis (Table S1).

Stomatal conductance is a strong regulator for photosynthetic response under water deficit

In order to verify if the gas exchange is limited by CO_2 diffusion limitations and/or a biochemical process, we performed a study about stomata's kinetic response to light transitions. The restriction of CO_2 uptake by lower CO_2 influx and net assimilation by g_s is a common adjustment under unfavorable plant growth conditions (MCAUSLAND *et al*., 2016). The balance between *A* and g_s directly affects plants' productivity WUE also can represent the grain yield/ water received or as total biomass/evapotranspiration at the field level (VADEZ *et al*., 2014). Thus, understanding this relationship represents a target for the improvement in crop performance (MCAUSLAND *et al*., 2016). During the steps changes *A* and g_s remained coupled in most treatments (Fig. 4). This response confirms that under drought stress, stomata are a major limiting factor (FLEXAS *et al*., 2004) for cashew plants under water deficit. From our results, 12 days of water deficit decreased g_s close to zero with a similar impact in *A* (Fig. 4). There was a clear difference between well-watered and plants under water deficit with fast and slow g_s increment, respectively. As in ambient conditions light changes rapidly and at successive times in a day, this is considered the most important environmental variable influencing both stomatal behavior and photosynthetic rate (MCAUSLAND *et al*., 2016). So, this response indicates that cashew plants have a fine control of the stomatal movements in order to preserve the internal hydric status in exchange to reduce the carbon gain. In most cases, slow stomatal increases represent reducing *A*, whereas slow g_s closure results in a decrease in WUE (BODNER; NAKHFOROOSH; KAUL, 2015). However, plants with slow stomatal closure, after 4 days of recovery had higher levels of $_i$WUE, and this this response was confirmed by *Ci/Ca*, as their lowest relation was also observed in recovery plants (Fig 7). A probable explanation for this could be the activation of the mechanisms for overall ¡WUE and carbon gain, as at this time g_s is not the limiting factor for *A* (Fig.4), (Fig. 7). In a study that characterized the impact of slow kinetics, a banana genotype with slow kinetic response also achieved the highest iWUE that was attributed to the period of the day, time, and light conditions(EYLAND *et al*., 2021).

Does water deficit affect stomata sensitivity to high CO_2?

We decided to complement stomatal characterization by step changes in CO_2 concentration (ambient to high) to better understand how stomata could limit *A* and WUE under water withdrawal. From this analysis, a very interesting response was observed. In most treatments, cashew plants became insensitive to CO_2 transitions, as they mostly *A* and g_s did not modify (Fig. 8). This is not an expected response, as in angiosperms stomata close when transferred to dark or high CO_2 (LIMA *et al*., 2018). Instead, the lack of stomata response to CO_2 is considered an exceptional observation, not a constituted condition, but caused by a combination of internal and environmental factors (MORISON, 1985). We did visualize this type of response in cashew plants, especially under very moderate water deficit (4 days) and recovery treatments (2 and 4 days) (Fig. 8 and S7). Even in ferns, a vascular plant used to be classified as high CO_2 insensitive due to key functional traits including sensitivity to elevated C_a and the stress hormone ABA to high CO_2, recent studies have pointed out that these plants do respond to this gas increment (FRANKS; BRITTON-HARPER, 2016; LIMA *et al*., 2019). Honestly, we recognize that this finding is very intriguing, raising the question of whether it could be an evolutive stomatal adjustment on cashew plants or an outlier response. Further investigation is required to analyze this doubt.

In addition, this unpredicted response also resulted in differences for iWUE. Under step change for CO_2 transitions, the highest values were observed in plants under water deficit and the lowest for plants after recovery treatments. Apart from this difference in response from the observations during light kinetics, recovered plants remained with iWUE almost unchanged as a result of *A* and g_s (Fig. 8 and S7). However, we agree that a deep understanding by correlation analysis for CO_2

response (*Ca*) varying its concentration with WUE will help in the comprehension of this response.

CONCLUSIONS

Overall, our observations reinforce the understanding that cashew plants are highly resilient to water deficit, likely through adaptive responses that include drought avoidance and tolerance mechanisms for prolonged periods of water withdrawal. Through a combination of physiological adjustments, these plants precisely manage water conservation sustaining growth processes, demonstrating a remarkable resilience to deal with hydric limitations. From our study, our analyses suggest new insights about stomatal conductance evidencing its role as a great controller of net CO_2 assimilation. In addition, cashew plants slowly respond to light and CO_2 transition as a mechanism to save water. We also noticed that reduced periods of water privation can positively regulate stomatal aperture, incrementing WUE and *A*. One of the most interesting findings highlighted from this work was the cashew plants' stomatal behavior under high CO_2 which differs from mostly angiosperms.

Statements & Declarations

Funding and Acknowledgments

This work was partially supported by the National Council for Scientific and Technological Development (CNPq), and The Ceará Foundation for Support of Scientific and Technological Development (FUNCAP). The authors gratefully acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support for PhD fellowship.

Conflict of interest

The authors declare no conflict of interest.

FIGURE LIST

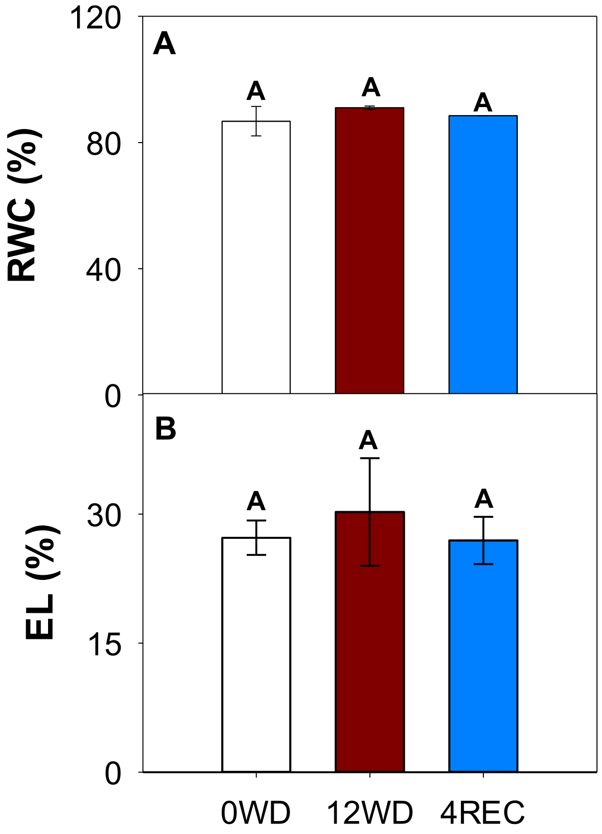


Figure 1. Physiological stress indicators of cashew plants under 12 days of water deficit and 4 days of recovery. Plants were analyzed under well-watered (0WD, white bar), 12 days of water deficit (12WD, red bar), and 4 days of rehydration recovery (4REC, blue bar). A, changes in relative water content (RWC %) and B, electrolyte leakage (EL %). Data are means of three replicates (n = 3) and the error bars show the standard deviation. Distinct capital letters represent significant differences by Tukey's test (*p≤0.05*).

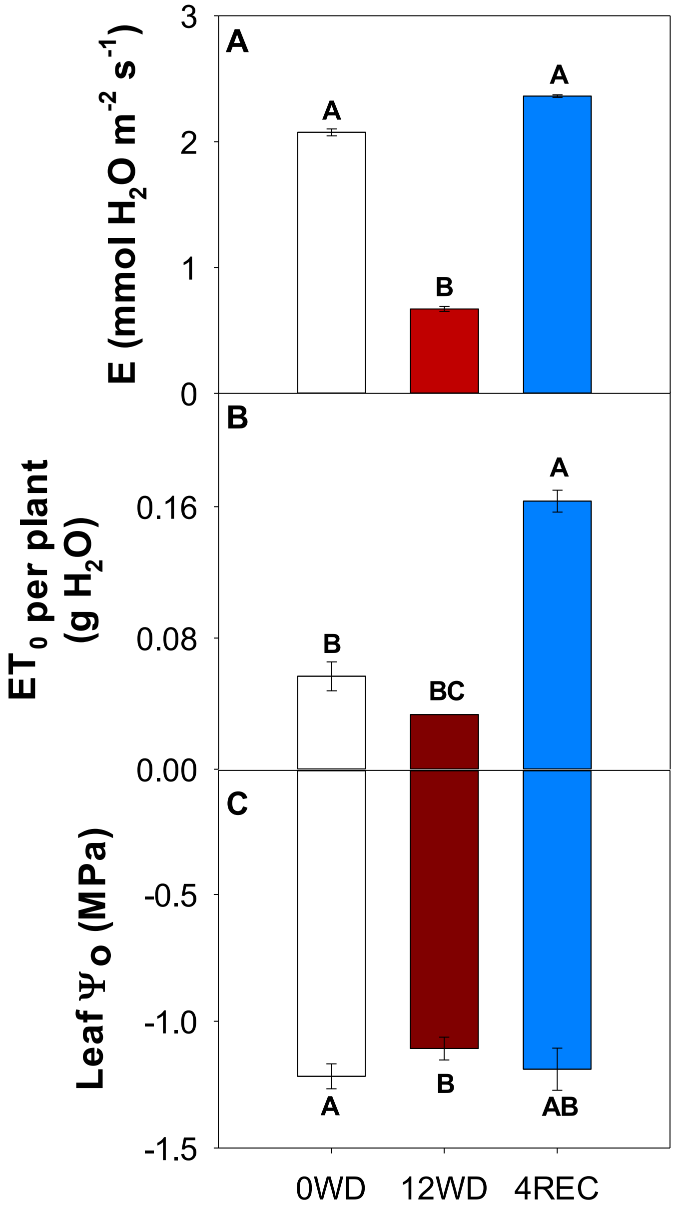


Figure 2. Transpiration, evapotranspiration, and leaf osmotic potential of cashew plants under 12 days of water deficit and 4 days of recovery. Plants were analyzed under well-watered (0WD, white bar), 12 days of water deficit (12WD, red bar), and 4 days of rehydration recovery (4REC, blue bar). A, leaf transpiration; B, total plant evapotranspiration (ET_0); and C, leaf osmotic potential (Ψo). Data are means of twenty eight replicates (n=28) for Leaf transpiration (E), and three replicates (n = 3) for total plant evapotranspiration (ET_0) and leaf osmotic potential (Ψo). Error bars show the standard deviation. Distinct capital letters represent significant differences by Tukey's test ($p \leq 0.05$).

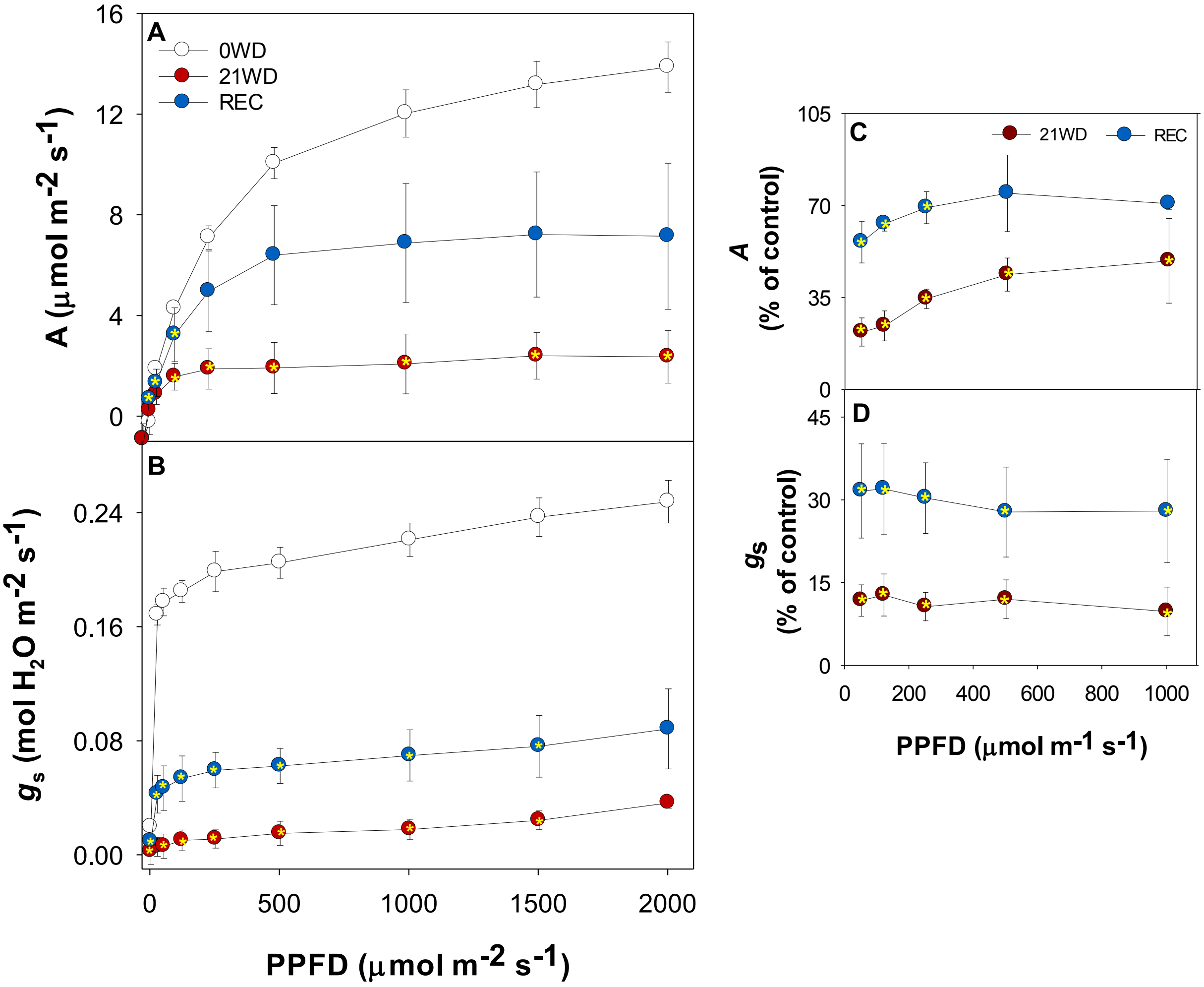


Figure 3. Net CO_2 assimilation (*A*) and stomatal conductance (g_s) curves in response to changes in photosynthetically active photon flux density (PPFD) in cashew leaves. Plants were subject to well-watered (0WD, white dots), 21 days of water deficit (21WD, red dots), and 5 days of rehydration recovery (REC, blue dots). A, photosynthetic light-response curves (*A*/PPFD); B, stomatal conductance light-response curves (g_s/PPFD); C, photosynthetic recovery light-response curves response expressed as % of well-watered plants (control); D, stomatal conductance recovery light-response curves response expressed as % of well-watered plants (control). The curves were performed under ambient CO_2 (400 ppm). The maximum value for PPFD (1000 μmol m^{-2} s^{-1}) was selected following the light saturation point for cashew plants (C, D). Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability ($p \leq 0.05$) compared to control (WW) in each treatment.

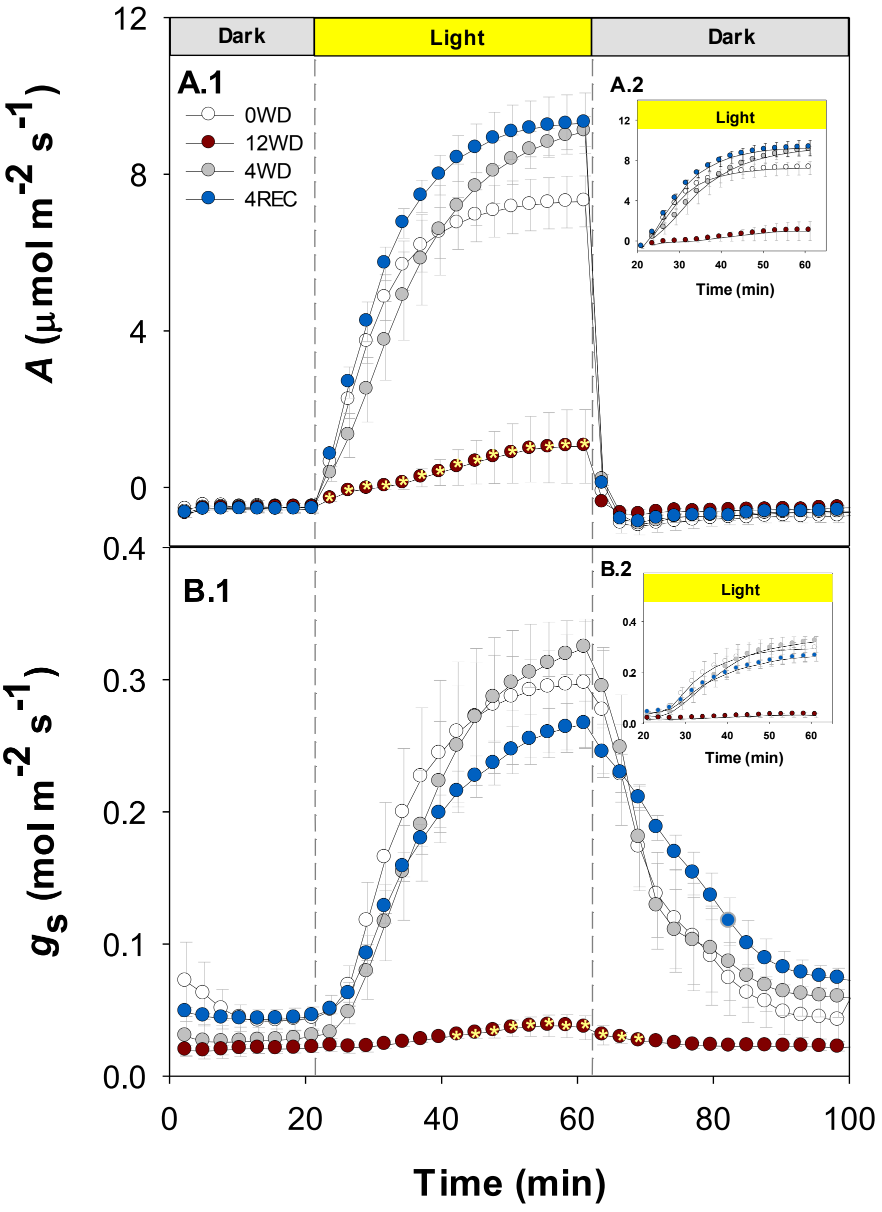


Figure 4. Net CO_2 assimilation, stomatal conductance in cashew leaf during light transitions. Plants were subject to well-watered (0WD, white dots), 4 and 12 days of water deficit (4WD and 12WD, grey and red dots, respectively) respectively, and 4 days of rehydration recovery (4REC, blue dots). A.1, Full Net CO_2 assimilation kinetic (A); A.2, Net CO_2 assimilation kinetic under light; B.1, Full stomatal conductance kinectic (g_s); B.2, stomatal conductance kinectic (g_s) under light . The kinetic series were measured under dark (0 μmol m^{-2} s^{-1}) and light (1000 μmol m^{-2} s^{-1}). The ambient CO_2 concentration was 400 ppm. Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability ($p \leq 0.05$) compared to control (0WD) in each treatment.

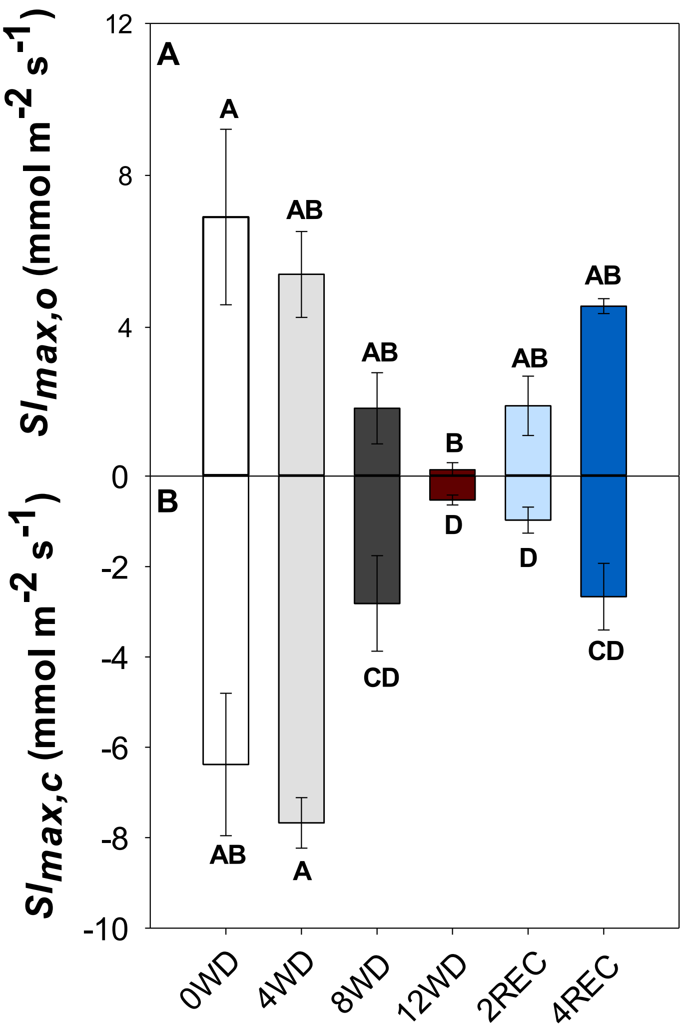


Figure 5. Maximum slope (*Slmax*) of stomatal conductance (g_s). A, for opening ($Sl_{max,o}$); and B, closing ($S_{lmax,c}$) in cashew leaf during a dark-to-light and light-to dark transitions. Plants were subject to well-watered (0WD, white bar), 4, 8, and 12 days of water deficit, respectively (4, 8,12WD, grey, black, and red bars, respectively), 2 and 4 days of rehydration recovery (2, 4REC light and dark blue bars, respectively). Distinct capital letters represent a significant difference between treatments under light (1000 μmol m^{-2} s^{-1}), whereas distinct minus letters represent significant difference under dark (0 μmol m^{-2} s^{-1}) by Tukey test at 5% probability ($P \leq 0.05$).

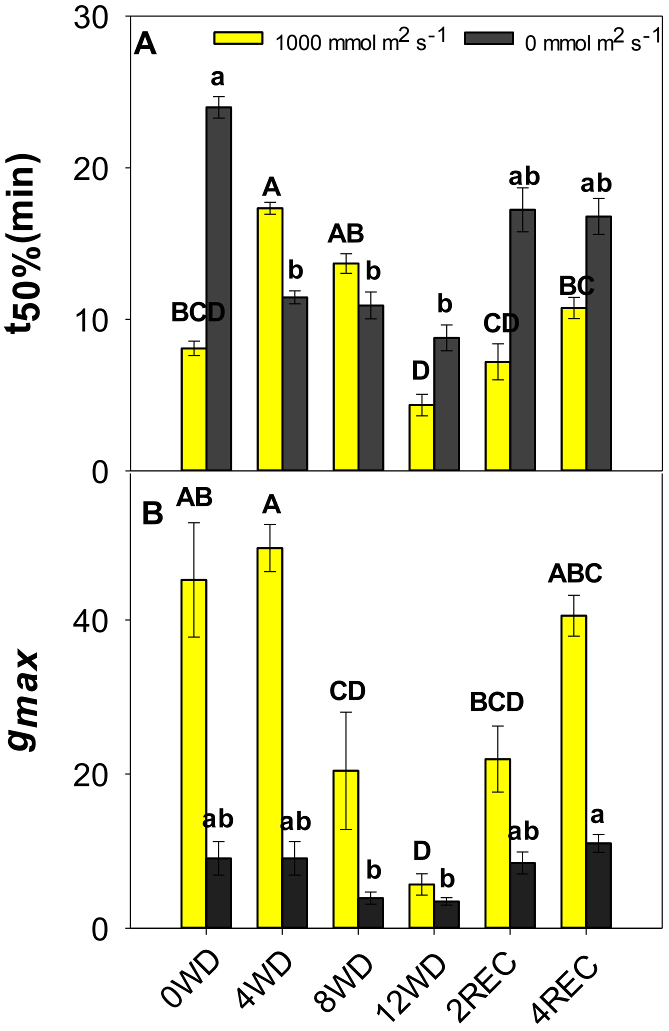


Figure 6. A, half-time steady state g_s (t50%); B, maximum theoretical stomatal conductance (*gmax*) in cashew leaf during dark-to-light transitions. Plants were subject to well-watered (0WD), 4, 8, and 12 days of water deficit, respectively (4, 8,12WD), 2 and 4 days of rehydration recovery (2, 4REC). Data are mean ± SE (n = 3). Distinct capital letters represent a significant difference between treatments under light (1000 μmol m^{-2} s^{-1}), whereas distinct minus letters represent significant difference under dark (0 μmol m^{-2} s^{-1}) by Tukey test at 5% probability ($p \leq 0.05$).

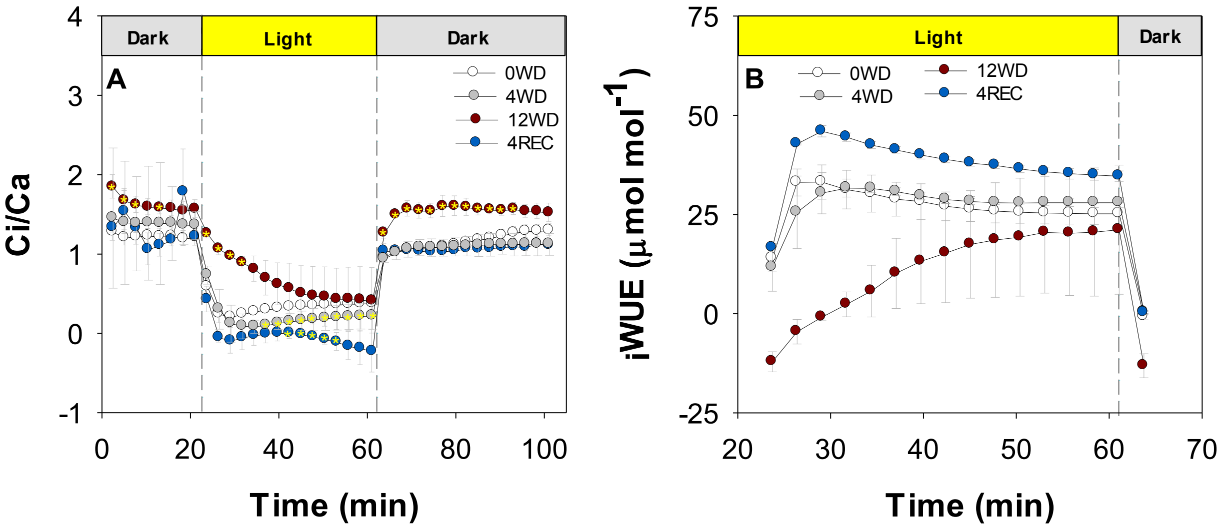


Figure 7. Ratio of substomatal and ambient CO_2 concentration, and $_i$WUE in cashew leaf during a dark-to-light and light-to dark transitions. Plants were subject to well-watered (0WD, white dots), 4 and 12 days of water deficit (4WD and 12WD, grey and red dots, respectively), and 4 days of rehydration recovery (4REC). A, Ratio of substomatal and ambient CO_2 concentration (Ci/Ca); B, intrinsic water use efficiency ($_i$WUE). The kinetic series were measured under dark (0 μmol m^{-2} s^{-1}) and light (1000 μmol m^{-2} s^{-1}). The ambient CO_2 concentration was 400 ppm. Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability (*p≤0.05*) compared to control (0WD) in each treatment.

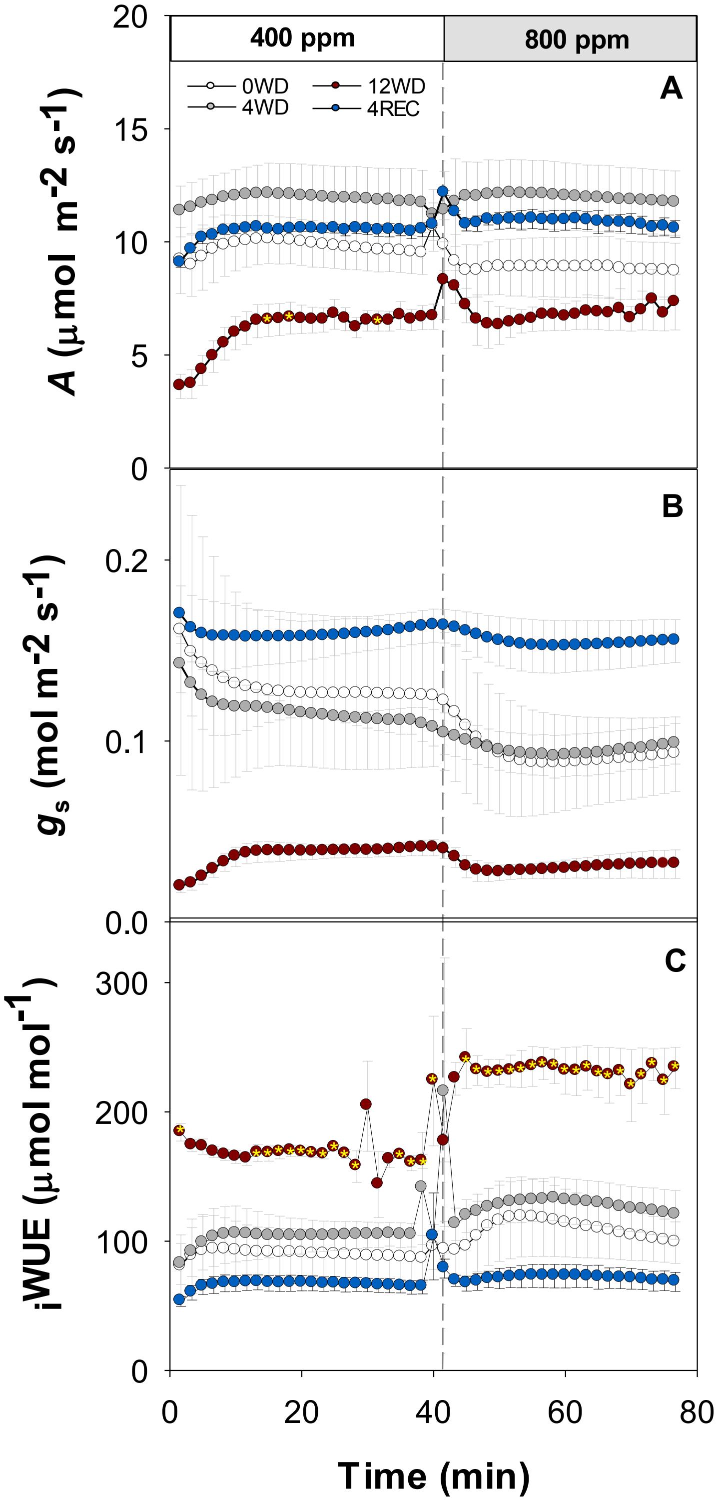


Figure 8. Net CO_2 assimilation, stomatal conductance, and WUE_i response in cashew leaf during increase in CO_2 concentration from 400 to 800 ppm. Plants were subject to well-watered (0WD, white dots), 4 and 12 days of water deficit (4WD and 12WD, grey and red dots, respectivvely), and 4 days of rehydration recovery (4REC, blue dots). A, Net CO_2 assimilation kinetic (A); B, stomatal conductance kinectic (g_s); C, intrinsic water use efficiency ($_i$WUE) over ambient to high CO_2. The kinetic series were measured under 1000 µmol m^{-2} s^{-1} PAR, ambient CO_2 (400 ppm) and high CO_2 (800 ppm). Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability (*p≤0.05*) compared to control (0WD) in each treatment.

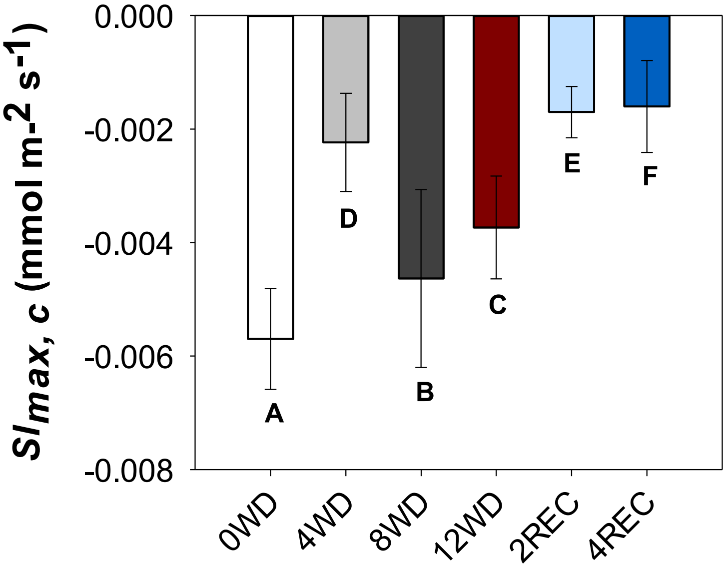


Figure 09. Maximum slope (Slmax) of stomatal conductance (g_s) for closing ($S_{lmax,c}$) in cashew leaf during increase in CO_2 concentration from 400 to 800 ppm. Plants were subject to well-watered (0WD, white bar), 4, 8, and 12 days of water deficit (4, 8,12WD, grey, black and red bars, respectively), 2 and 4 days of rehydration recovery (2, 4REC light and dark blue bars, respectively). Data are mean ± SE (n = 3). Distinct capital letters represent a significant difference between treatments under CO_2 concentration from 400 to 800 ppm. by Tukey test at 5% probability ($p \leq 0.05$).

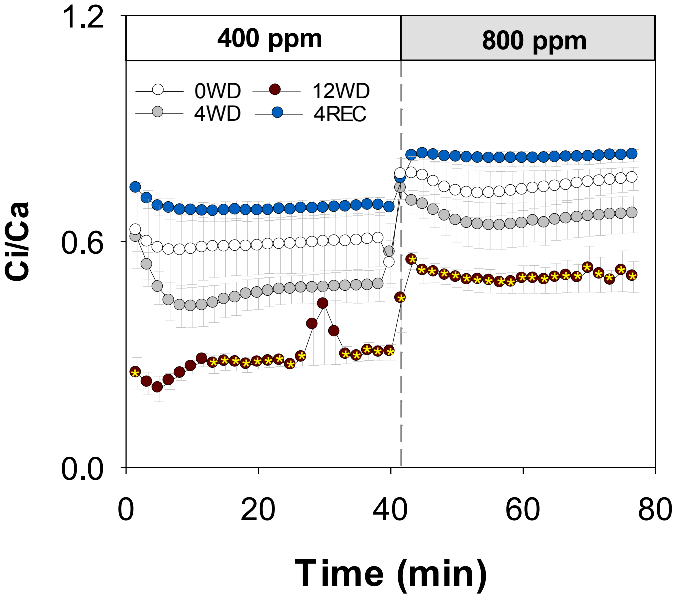


Figure 10. Ratio of substomatal and ambient CO_2 concentration (Ci/Ca) in cashew leaf during increase in CO_2 concentration from 400 to 800 ppm. Plants were subject to well-watered (0WD, white dots), 4 and 12 days of water deficit (4WD and 12WD, grey and red dots, respectively), and 4 days of rehydration recovery (4REC, blue dots). The kinetic series were measured under 1000 μmol m^{-2} s^{-1} PAR, ambient CO_2 (400 ppm) and high CO_2 (800 ppm). Data are mean ± SE (n = 3). Yellow asterisks

represent significant differences by Student's t test 5% probability (*p≤0.05*) compared to control (0WD) in each treatment.

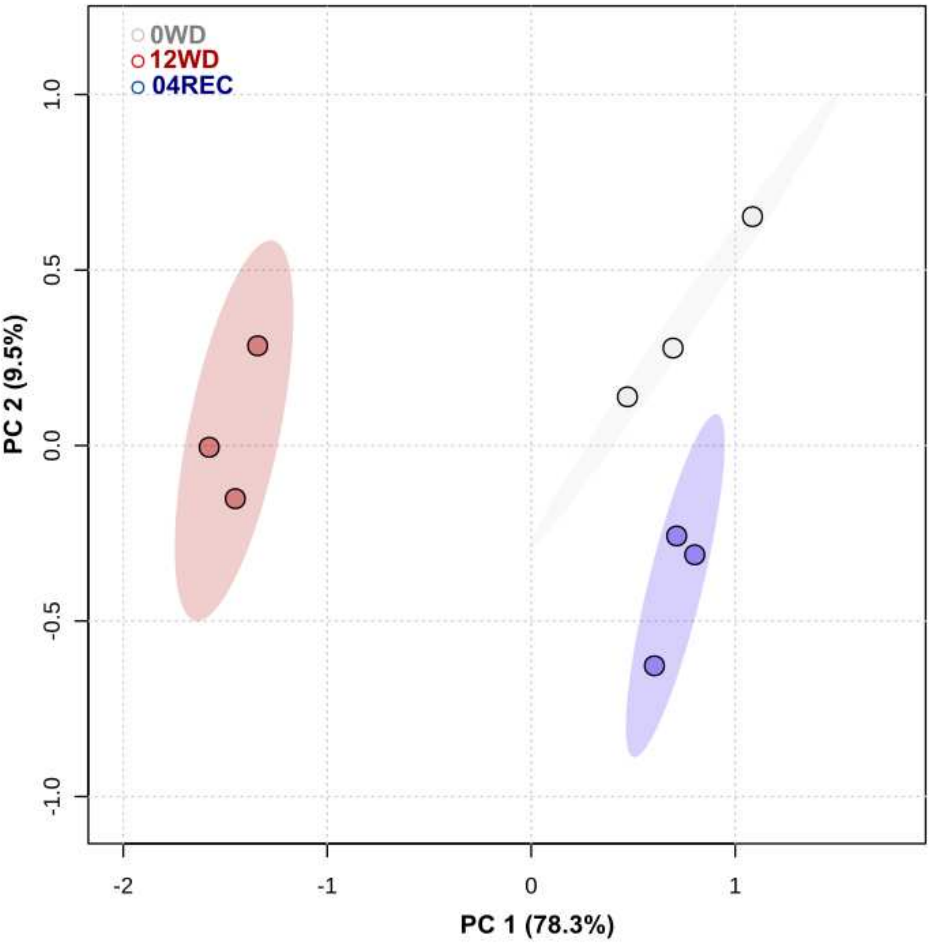
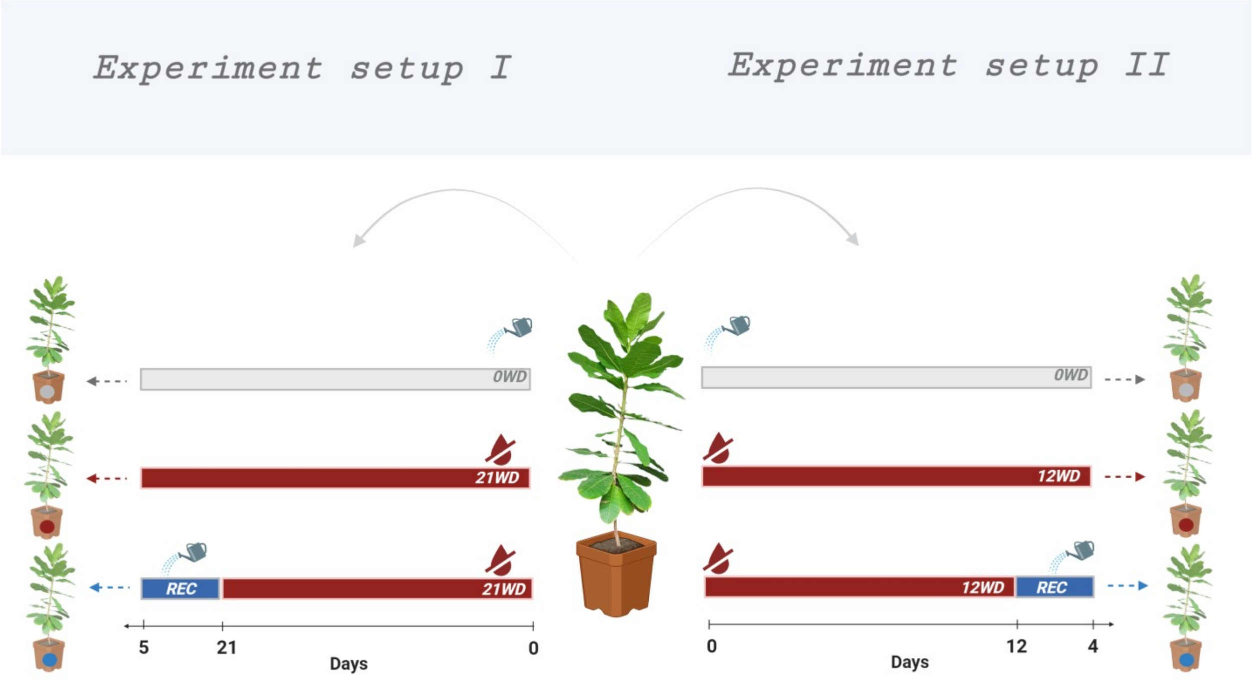


Figure 11. Principal component analysis (PCA) of cashew leaf physiological stress indicators and stomatal and photosynthetic kinetics responses during light transitions data. The analysis was performed using MetaboAnalyst platform. (n = 3).



*0WD: well-watered; 21WD: 21 days of water deficit; REC: 5 days of recovery.

*0WD: well-watered; 12WD: 12 days of water deficit; 4REC: 4 days of recovery.

Figure S1. Experiment I and II setup. In the experiment I, ccashew plants with up to ± ten expanded leaves (45 days old) were divided into three groups according to the hydric regime supplied: (0WD) control, non-water deficit; (21WD) twenty one days of water deficit; (REC) five days of recovery with water rehydration supply. The time for water deficit was stabilized according to previous experiments for the optimal timing of severe reversible stress. From this experiment we performed net photosynthesis curves in response to changes in photosynthetically active photon flux (*A/PPFD*) in fully expanded leaves. For the experiment II, cashew plants with up to ± forty expanded leaves (5 months old after transplant) were maintained in similar growth conditions were divided into six groups according to the hydric regime supplied: (0WD) control, non-water deficit; (4WD) four days of water deficit; (8WD) eight days water deficit; (12WD) twelve days of water deficit; (2REC) two days of recovery with water rehydration supply; (4REC) four days of recovery with water rehydration supply. We chose the most contrasting hydric regimes for the main results: 0WD, 12WD, and 4REC. From this experiment, we performed light and CO_2 kinetic curves.

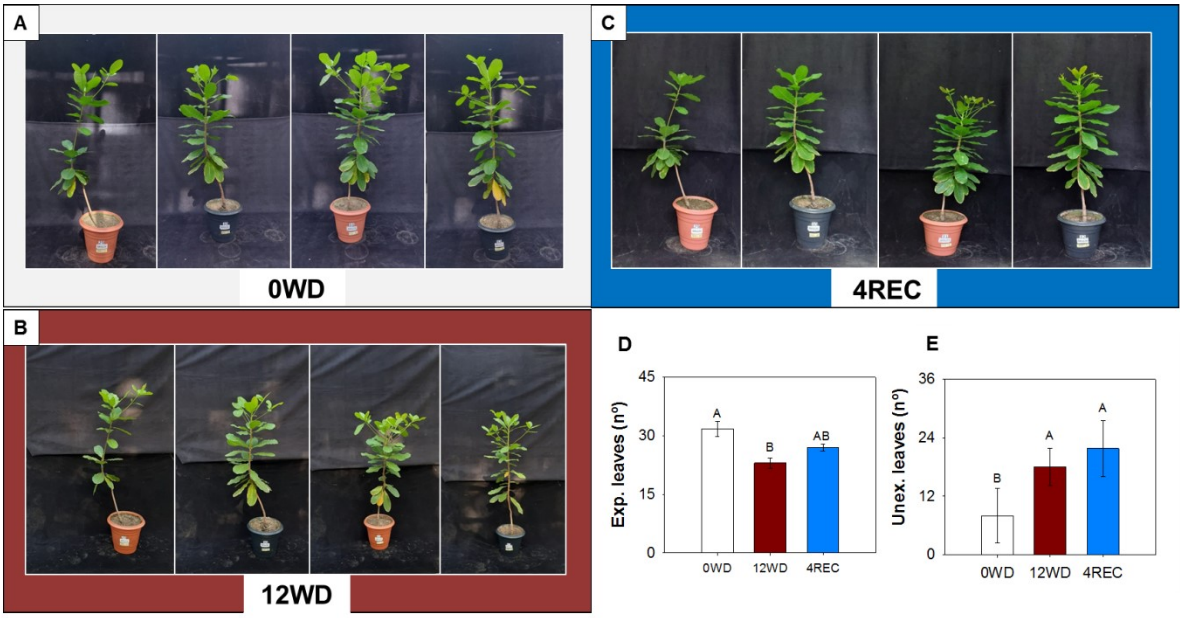


Figure S2. Morphological aspects of cashew plants after treatments. A, well-watered (0WD, white bar), B, 12 days of water deficit (12WD, red bar), C, 4 days of rehydration recovery (4REC, blue bar), D, number of expanded leaves (fully developed), and E, number of unexpanded leaves (underdeveloped). The pictures are representative of four independent replicates (n=4) for each treatment. Error bars show the standard deviation. Distinct capital letters represent significant differences by Tukey's test ($p \leq 0.05$).

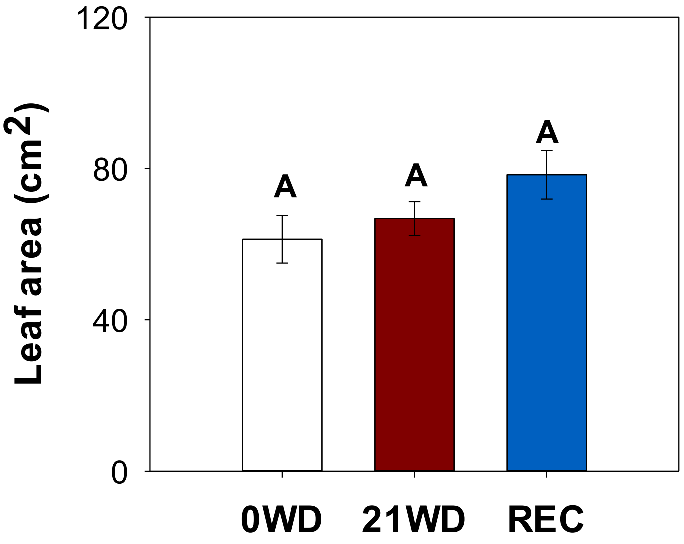


Figure S3. Changes in leaf area (cm^2) of cashew plants after treatments. Plants were subject to well-watered conditions (0WD, white bar), 21 days of water deficit (21WD, red bar), and 5 days of rehydration recovery (REC, blue bar). Data are mean of three replicates (n = 5) and the error bars show the standard deviation. Distinct capital letters represent significant differences by Tukey's test ($p \leq 0.05$).

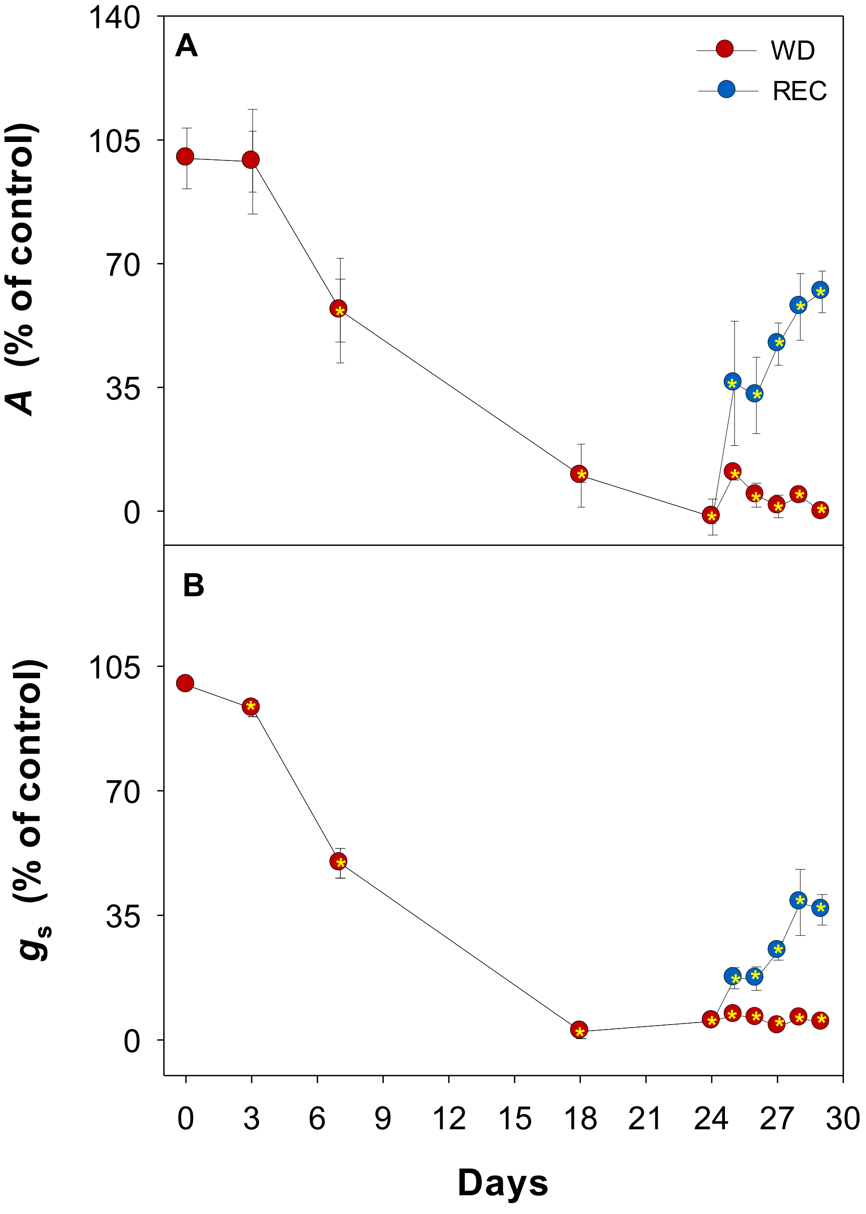


Figure S4. Relative Net CO_2 assimilation and stomatal conductance of cashew plants under water deficit and recovery steadystates measured among days (29 days). Plants were subject to well-watered (WW, blue dots), 21 days of water deficit (WD, red dots), and 5 days of rehydration recovery (REC). A, Relative Net CO_2 assimilation expressed as percentage of well-watered plants (control) (*A*); B, stomatal conductance expressed as percentage of well-watered plants (control) (g_s). Measurements were performed under 1000 μmol m^{-2} s^{-1} PAR and ambient CO_2 (400 ppm). Data are mean of three replicates (n = 5) and the error bars show the standard deviation. Distinct capital letters represent significant differences by Tukey's test (*p≤0.05*). Yellow asterisks represent significant differences by Student's t test 5% probability (p≤0.05) compared to control (WW) in each treatment. This figure was adapted from Capelari *et al*. (2021).

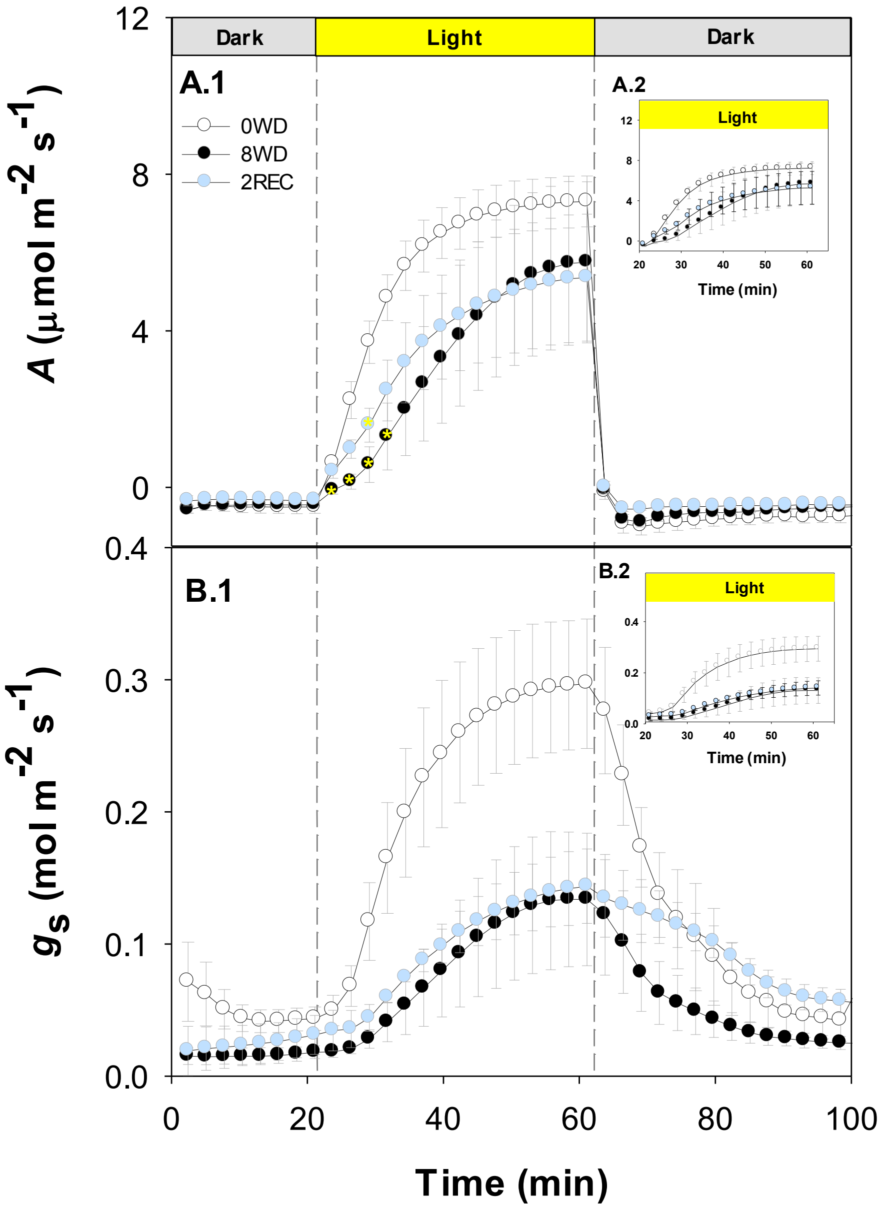


Figure S5. Net CO_2 assimilation, stomatal conductance in cashew leaf during light transitions. Plants were subject to well-watered (0WD, white dots), 8 days of water deficit (8WD, black dots), and 2 days of rehydration recovery (2REC, light blue dots). A.1, Full Net CO_2 assimilation kinetic (*A*); A.2, Net CO_2 assimilation kinetic under light; B.1, Full stomatal conductance kinectic (g_s); B.2, stomatal conductance kinectic (g_s) under light. The kinetic series were measured under dark (0 μmol m^{-2} s^{-1}) and light (1000 μmol m^{-2} s^{-1}). The ambient CO_2 concentration was 400 ppm. Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability ($p \leq 0.05$) compared to control (0WD) in each treatment.

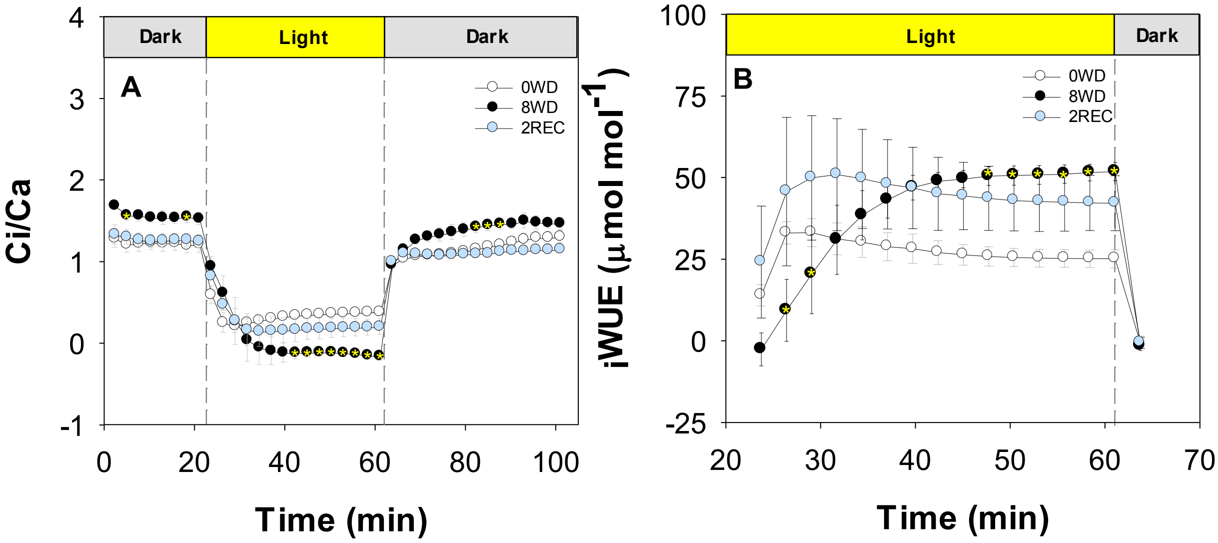


Figure S6. Ratio of substomatal and ambient CO_2 concentration, and $_i$WUE in cashew leaf during a dark-to-light and light-to dark transitions. Plants were subject to well-watered (0WD, white dots), 8 days of water deficit (8WD, black dots), and 2 days of rehydration recovery (2REC, light blue dots). A, Ratio of substomatal and ambient CO_2 concentration (Ci/Ca); B, intrinsic water use efficiency ($_i$WUE). The kinetic series were measured under dark (0 μmol m^{-2} s^{-1}) and light (1000 μmol m^{-2} s^{-1}). The ambient CO_2 concentration was 400 ppm. Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability (*p≤0.05*) compared to control (0WD) in each treatment.

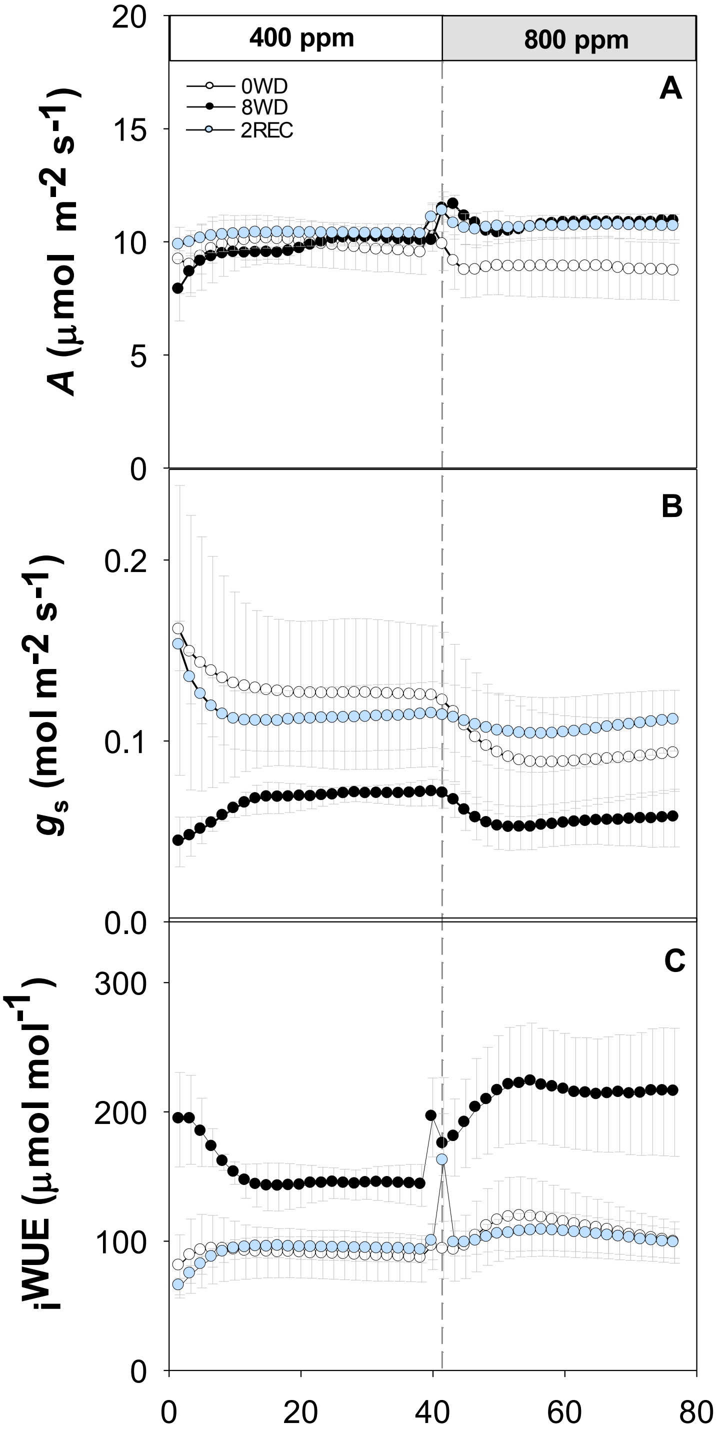


Figure S7. Net CO_2 assimilation, stomatal conductance, and $_i$WUE response in cashew leaf during increase in CO_2 concentration from 400 to 800 ppm. Plants were subject to well-watered (0WD, white dots), 8 days of water deficit (8WD, black dots), and 2 days of rehydration recovery (2REC, light blue dots). A, Net CO_2 assimilation kinetic (A); B, stomatal conductance kinectic (g_s); C, intrinsic water use efficiency ($_i$WUE) over ambient to high CO_2. Plants were subject to well-watered (0WD), 8 days of water deficit (8WD), and 2 days of rehydration recovery (2REC The kinetic series were measured under 1000 μmol m^{-2} s^{-1} PAR, ambient CO_2 (400 ppm) and high CO_2 (800 ppm). Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability (*p≤0.05*) compared to control (0WD) in each treatment.

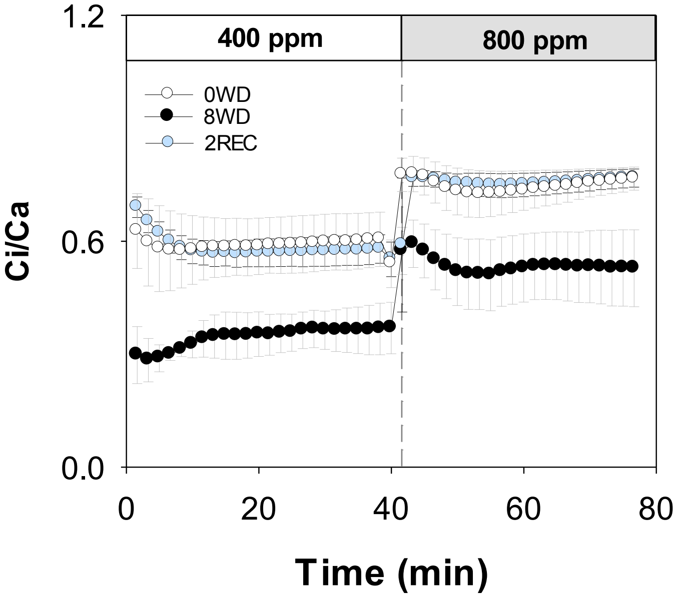


Figure S8. Ratio of substomatal and ambient CO_2 concentration (Ci/Ca) in cashew leaf during a dark-to-light and light-to dark transitions. Plants were subject to well-watered (0WD, white dots), 8 days of water deficit (8WD, black dots), and 2 days of rehydration recovery (2REC, light blue dots). The kinetic series were measured under 1000 μmol m^{-2} s^{-1} and ambient CO_2 (400 ppm). Data are mean ± SE (n = 3). Yellow asterisks represent significant differences by Student's t test 5% probability (*p≤0.05*) compared to control (0WD) in each treatment.

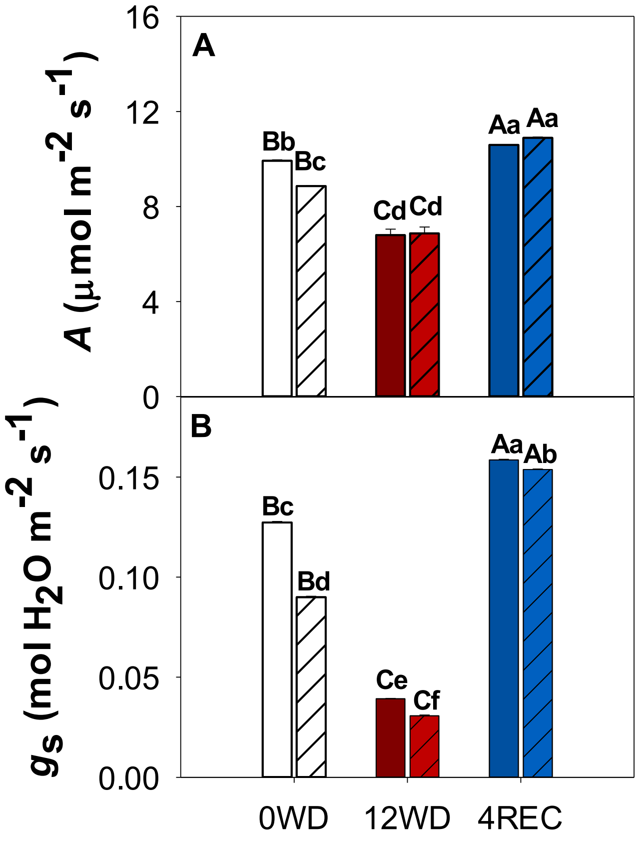


Figure S9. Steady state of *A*, Net CO_2 assimilation (A); B, stomatal conductance (g_s). Plants were analyzed under well-watered (0WD, white bars), 12 days of water deficit (12WD, red bars), and 4 days of rehydration recovery (4REC, blue bars). The steady states were measured under 1000 μmol m^{-2} s^{-1} PAR, A, B ambient CO_2 (400 ppm); or C, D high CO_2 (800 ppm) after exposure to light until *A* and g_s stabilization. Data are mean of fifteen replicates (n = 15) and the error bars show the standard deviation. Dashed bars represent high CO_2 concentration (800 ppm). Distinct capital letters represent significant differences between water regimes in the same treatment, whereas distinct minus letters represent significant difference between water regimes comparing ambient (400 ppm) to high (800 ppm) CO_2 concentrations by Tukey's test ($p \leq 0.05$).

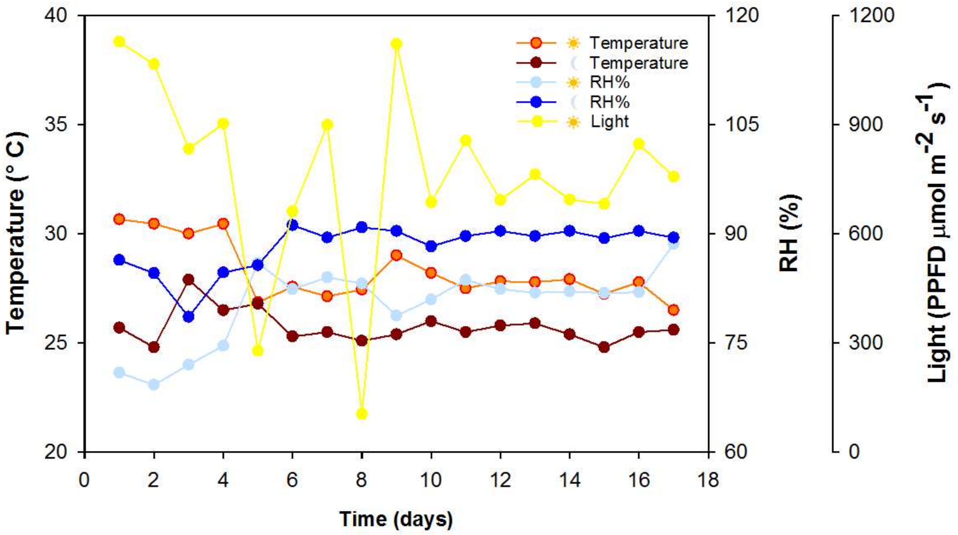


Figure S10. Environmental average of temperature (ºC), relative humidity (RH %), and light (PPFD µmol photons m-^{2}s-1) during day and night over seventeen days of the experiment under greenhouse conditions.

TABLES

Table S1: Photosynthetic parameters from light-response curves in cashew plants (BRS226) under WW (well-watered), WD (water deficit 21 days), and REC (5 days of rehydration recovery). Data are mean ± SE (n = 3). Asterisks represent significant differences by Student's t test 5% probability ($p \leq 0.05$) compared to control (0WD) in each treatment.

Parameters	**0WD**	**21WD**	**REC**
*Amax*_Farquhar *et al*. 1980 (μmol m^{-2} s^{-1})	16.47	5.97*	9.02*
Rd_Farquhar *et al*. 1980 (μmol m^{-2} s^{-1})	1.09	0.77	1.25
1/Φ Farquhar *et al*. 1980 (μmol m^{-2} s^{-1})	19.25	15.83	22.51
*LCP*_Farquhar *et al*. 1980 (μmol m^{-2} s^{-1})	16.78	19.22*	13.34
*LSP*_Farquhar *et al*. 1980 (μmol m^{-2} s^{-1})	549.38	287.93*	216.12*
*Jmax*_Harley 2015 (μmol m^{-2} $s^{-1)}$	131.74	117.29	62.35*

**Amax*: light-saturated net CO_2 assimilation rate (reference value); Rd: Rate of non-photorespiratory CO_2 release; *1/Φ :* light use efficiency; LCP: light compensation point; *LSP*: light saturation point; and *Jmax*: maximum rate of photosynthetic electron transport.

5 CONCLUSIONS

Cashews (*Anacardium occidentale* L.) are a well-adapted plant species in many regions worldwide, in the northeast of Brazil; it is mostly cultivated in semiarid regions, which usually face multiple limited conditions. Although the literature cites a great number of studies among these species, there is not a concern about how cashew plants triggers defense response mechanism to protect against stress. In addition, still a lack of studies which have tried to understand how cashew plants may adjust it photosynthetic capacity when live these type of process.

From this study, we started to get insights into how cashew plants deal with high temperatures, water deficit, and salinity. We noticed that these plants have a very coordinator antioxidant responses triggered under high temperature and when under water deficit. In addition, we first observed the very remarkable stomata regulation mechanism of cashew plants under distinct water regimes. These results highlight this plant species as a powerful vegetal model of study in the actual climate change scenario, its potential for crop breeding, and photosynthetic knowledge about how plants may adjust their water use efficiency under unfavorable environmental conditions.

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ATTACHMENT A – SCIENTIFIC PUBLICATIONS RELATED TO THE THESIS'S THEME THROUGHOUT THE DOCTORATE PERIOD

Journal of Plant Physiology

High temperatures favor antioxidant protection and display contrasting interactions with water deficit and salinity in cashew plants

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	Anacardium occidentale L; Oxidative metabolism; Combined stress; Water Deficit; Salt.
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	Fabricio Eulálio Leite Carvalho, PhD
	Joaquim Albenisio Gomes Silveira, PhD
Manuscript Region of Origin:	BRAZIL
Abstract:	High temperatures (HT), an important issue in thermotolerant plants, favors metabolism and physiological processes. However, little is known how HT interacts with other abiotic factors in response to redox metabolism. In this study, we employed a thermotolerant plant, cashew (Anacardium occidentale L.) to test the hypothesis that HT favors antioxidant responses with different mechanisms to deal with water deficit (WD) and salinity. In the first experiment, plants were exposed to progressive HT (25 ºC – 50 ºC) in a growth chamber. In the second experiment, plants were exposed to WD (20 days of water withdrawal) and salinity (NaCl 100 mM for 15 days) under control (25 ºC) and HT (45 ºC) for 12 hours. Progressive temperatures raised stomatal conductance (gs) and transpiration (E) until 45 ºC followed by decrease at 50 ºC, maintaining their hydric status unchanged. Progressive HT augmented TBARS and H2O2, followed by ASC and GSH redox state increases (35 ºC – 50 ºC). In parallel, progressive HT stimulated catalases (CAT) and superoxide dismutases (SOD) activities. Interestingly, the HT + WD combined effects stimulated E and lipid peroxidation compared to HT + Salt. Moreover, HT + WD combined treatment induced increase in the GSH redox state by WD influence, not affecting ASC oxidation. In contrast, the HT + Salt drastically decreased the ASC redox state by HT influence. Our results show that cashew antioxidant system effectiveness is associated to HT represented by reduced ascorbate, reduced glutathione, CAT, and SOD activities. However, the ASC and GSH oxidation are differently triggered in response to HT, WD and salinity combined treatments.
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ATTACHMENT B – ARTICLE PUBLISHED IN COLLABORATION INVOLVING MOLECULAR AND PHYSIOLOGICAL RESPONSES TO WATER DEFICIT AND RECOVERY DISPLAYED BY CASHEW PLANTS

plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Transcriptional profiling and physiological responses reveal new insights into drought tolerance in a semiarid adapted species, Anacardium occidentale

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Keywords
Anacardium occidentale; transcriptome; water deficit; recovery; abiotic stress; differential expression; cashew tree.

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Editor
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Received: 4 March 2021; Accepted: 5 May 2021

doi:10.1111/plb.13312

ABSTRACT

- Water stress affects plant performance at various organisational levels, from morphological to molecular, with a drastic drop in crop yield. Integrative studies involving transcriptomics and physiological data in recognized tolerant species are appropriate strategies to identify and understand molecular and functional processes related to water deficit tolerance. The cashew tree (*Anacardium occidentale*) is a species naturally adapted to environments with low water availability associated with adverse conditions such as heat, high radiation and salinity.
- We used an integrative strategy, combining classical physiological measurements with high throughput RNA-seq to understand the main adaptive mechanisms of cashew to water deficit followed by recovery.
- Physiological analyses indicate that young cashew plants display typical isohydric behaviour. They first exhibit rapid stomatal closure, followed by CO_2 assimilation, thus preserving the relative water content, membrane integrity and photosystem II activity. Differential expression was observed in 1733 genes from plant leaves exposed to water deficit stress for 26 days. Among them, 705 were upregulated and 1028 were downregulated. After rewatering, 1330 (76.7%) genes returned to their basal expression level.
- Transcriptional, combined with physiological data, reveal that cashew plants display high phenotypic plasticity and resilience to acute water deficit, and do not activate senescence pathways. A series of genes/pathways and processes involved with drought tolerance in cashew are evidenced, particularly in carbon metabolism, photosynthesis and chloroplast homeostasis.

INTRODUCTION

Drought is defined as an imbalance between water availability in the soil and plant transpiration demand, resulting in changes in plant water status and decreased growth and productivity (Tardieu *et al.* 2019). Over the last few decades, water stress in agriculture has increased in both intensity and frequency in many areas worldwide (Marengo 2008). As a result, crop productivity has been drastically affected, leading to significant yield loss. The combination of water deficit and extreme temperatures results in decreased food production and quality, representing a serious threat to world agriculture (IPCC 2014). In Brazil, the most significant drought over the last 50 years occurred between 2012–2013. It caused a loss of US$ 1.6 billion to the ten most important crops in the country (Instituto Brasileiro de Geografia e Estatística–IBGE 2016), mainly in the northeast of Brazil, where drought occurs with high frequency and intensity (Marengo *et al.* 2008). Drought causes cell dehydration and loss of turgor, which directly affects the fundamental process of cell elongation and plant growth. It triggers stomatal closure and compromises CO_2 photosynthetic assimilation, causing changes in the redox balance inside the cells, affecting critical physiological processes, such as photosynthesis and respiration, leading to potential damage to cell components, such as proteins, phospholipids and pigments. At the whole plant level, critical decreases in plant water potential increase the risk of xylem cavitation, hydraulic failure and mortality (Hochberg *et al.* 2017). To cope with drought stress, plants evolve different strategies to resist or tolerate critical low water potentials, including morphological, physiological and molecular adjustments. However, plant adaptation mechanisms vary largely among species, genotypes, plant developmental stages and in response to the progress of drought through time (Fang & Xiong 2015).

In general, plants respond to drought at an early stage of the stress development by resisting or avoiding critical decreases in water potential to retain normal physiological processes (Nolan *et al.* 2017). Plants may increase water uptake through investments in the root system and water

ATTACHMENT C – OTHER NON-RELATED PUBLICATIONS TO THESIS'S THEME IN COLLABORATION THROUGHOUT THE DOCTORATE PERIOD

Plant Cell, Tissue and Organ Culture (PCTOC) (2019) 139:139–153
https://doi.org/10.1007/s11240-019-01672-w

ORIGINAL ARTICLE

Photoperiod modulates growth, morphoanatomy, and linalool content in *Lippia alba* L. (Verbenaceae) cultured in vitro

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Received: 17 June 2019 / Accepted: 8 August 2019 / Published online: 12 August 2019

Abstract

Interactions between circadian clock regulation and metabolic responses are believed to explain the importance of rhythmic behavior in plant growth and survival. *Lippia alba* is an important species because of the medicinal properties of its essential oil extract. The objective of this work was to evaluate the effect of photoperiod on anatomy, growth, essential oil profile, and the expression of genes related to the synthesis of monoterpenes, sesquiterpenes, and the circadian clock in *L. alba* grown in vitro. The plants were cultured in vitro under different photoperiods (4, 8, 16, and 24 h of light) and irradiance of 41 µmol m^{-2} s^{-1}. After 40 days of culture, results showed that *L. alba* presented high physiological plasticity under different photoperiods, with improved performance when exposed to continuous light. The best growth; anatomical organization of the mesophyll, stem, roots, and bundles; amount of photosynthetic pigments; photosynthetic rate; and protein synthesis occurred under a photoperiod of 24 h. The biosynthesis of linalool, the major compound, was increased under the 24-h photoperiod, possibly due to reduced geraniol synthesis. These findings allow a better understanding of how photoperiod acts in the regulation of primary and secondary metabolism, and especially with regard to the composition of essential oils.

Key message

Photoperiod modulates primary metabolism, growth, morphoanatomy, photosynthesis, and essential oil content in the medicinal plant *Lippia alba* cultured in vitro under 4, 8, 16 or 24 h of light.

Keywords Circadian rhythm · Geraniol synthase · Internal clock · Medicinal plant · Photosynthesis

Introduction

The photoperiod is established by the rotation of the Earth around its own axis and around the sun, and impacts significantly the metabolism of most living beings (Serrano-Bueno et al. 2017). In plants, several process are controlled by photoperiod, like growth, flowering induction, cold tolerance and regulation of the circadian clock (Bordage et al. 2016; Barros et al. 2017; Shin et al. 2017; Maurya and Bhalerao 2017; Mahmud et al. 2018; Triozzi et al. 2018).

Interactions between the circadian clock, primary metabolism, and signaling are thought to explain the importance of rhythmic behavior for plant growth and survival (Harmer et al. 2000; Dodd et al. 2005; Shin et al. 2017). Despite a variety of reports involving the circadian regulation of primary metabolism pathways in plants, it remains to be

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Communicated by Mohammad Faisal.

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Environmental and Experimental Botany 149 (2018) 59–69

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Antioxidant protection and PSII regulation mitigate photo-oxidative stress induced by drought followed by high light in cashew plants

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ARTICLE INFO

Keywords:
Anacardium occidentale
Excess energy
NPQ
Photosynthesis
Water deficit

ABSTRACT

We tested the hypothesis that effective antioxidant and photoprotective mechanisms are able to avoid photodamage induced by prolonged water deficit (WD) followed by high light (HL). We employed cashew plants (*Anacardium occidentale* L.), a semiarid adapted species, as a model plant. WD-plants exposed to HL did not show alterations in maximum quantum efficiency of photosystem II (Fv/Fm), cellular integrity, H_2O_2 and thiobarbituric acid reactive species (TBARS) contents, evidencing that they did not suffered photoinhibition and oxidative stress. These responses were associated with increases in ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities, glutathione (GSH) oxidation and ascorbate (ASC) synthesis. This effective oxidative protection occurred in parallel to strong decrease in photosystem II and I (PSII and PSI) activities, increase in heat dissipation (qE), which was related to enhancement in cyclic electron flux. These favorable photoprotective changes were associated with efficient water use in response to HL, all contributing to avoid excess energy in chloroplasts of drought-exposed leaves. These protective features were associated with a tight regulation in D1 protein accumulation during HL, contributing to avoid reactive oxygen species over-accumulation and a subsequent effective PSII recovery during darkness. Our results indicate that cashew plants are able to avoid photoinhibition, tolerating extreme conditions of drought combined with HL. The displayed mechanisms involve essentially integrated responses to balance energy input and output, avoiding oxidative stress.

1. Introduction

Abiotic stress in tropical semiarid regions involves diverse limiting factors for plant growth and development, such as drought, salinity, heat and excessive light. In such regions these constraints generally persist for a long time during the year, affecting severely plant growth and survival. Plants highly adapted to these conditions have developed specific mechanisms of acclimation that are associated with maintaining photosynthetic capacity (Adams et al., 2013; Demmig-Adams et al., 2017, 2015). Some of these evergreen species can trigger acclimation mechanisms when exposed simultaneously to water deficit (WD) and high light (HL) (Suzuki et al., 2014). These strategies involve a more efficient water use (Flexas et al., 2013), maintaining high CO_2 assimilation under low stomatal aperture (Galmés et al., 2013, 2006) and triggering several other photoprotective mechanisms (Suzuki et al., 2014).

These conspicuous mechanisms might allow semiarid adapted plants to grow under extreme environmental conditions, where other species would not survive (Adams et al., 2013; Demmig-Adams et al., 2017, 2015). Numerous mechanisms related to plant defense against

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Abbreviations: ASC, reduced ascorbate; C_i, intercellular CO_2 partial concentration; DAB, diaminobenzidine; E, leaf transpiration; ETR, electron transport rate from PSII; F_v/F_m, maximum quantum yield of PSII; g_s, stomatal conductance; GSH, reduced glutathione; NPQ, non-photochemical quenching; P_N, CO_2 assimilation rate; PPFD, photosynthetic photon flux density; PSII, photosystem II; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; WD, water deficit; WW, well-watered; WUE, water use efficiency; ΦPSII, effective quantum yield of PSII; ΦPSI, effective quantum yield of PSI

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https://doi.org/10.1016/j.envexpbot.2018.02.001
Received 23 October 2017; Received in revised form 17 January 2018; Accepted 4 February 2018

ATTACHMENT D – OTHER SCIENTIFIC ACTIVITIES DEVELOPED THROUGHOUT THE DOCTORATE PERIOD

UNIVERSIDADE FEDERAL DO CEARÁ
CENTRO DE CIÊNCIAS
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GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

LUIS FLÁVIO MENEZES ROCHA JÚNIOR

SPATIO-TEMPORAL REDOX CHANGES IN RICE LEAVES EXPOSED TO HIGH LIGHT

FORTALEZA

2022

UNIVERSIDADE FEDERAL DO CEARÁ
CENTRO DE CIÊNCIAS
DEPARTAMENTO DE BIOQUÍMICA E BIOLOGIA MOLECULAR
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

MAIANY ALVES PATRIOTA

RELATIONSHIP BETWEEN CATALASE AND GLYCOLATE OXIDASE ACTIVITIES IN RESPONSE TO CONTRASTING PHOTORESPIRATORY CONDITIONS INDUCED BY DIFFERENT LIGHT INTENSITIES

FORTALEZA
2022

ABSTRACT - PLANT PHYSIOLOGY

SINGLE HEAT IS MORE EFFECTIVE TO TRIGGER ANTIOXIDANT MECHANISMS THAN WATER DEFICIT UNDER MODERATE OR HIGH TEMPERATURES IN ANACARDIUM OCCIDENTALE PLANTS

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Cashew plants (Anacardium occidentale) are tolerant to extreme semiarid conditions such as hot, drought, salinity, and excess light. Previously, we demonstrated that high temperatures (HT) induce antioxidant mechanisms to cope with salt stress conditions for this specie. Here, we hypothesized that cashew plants display a more effective antioxidant defense response to water deficit (WD) under HT. Plants were exposed or not to WD for 20 days under greenhouse conditions. Then, these plants were subjected or not to HT (42 oC) for 12h (low light). As stress indicators, electrolyte leakage in leaves was not affected by any treatment, whereas relative water content was slightly decreased by WD. In contrast, hydrogen peroxide and lipid peroxidation were similarly increased by HT and WD under both temperatures. Interestingly, despite these similar oxidative responses, only HT was capable to produce an antioxidant response with a strong increase in CAT, APX, and SOD isoform activities with consumption of reduced ascorbate. Moreover, WD singly or in

combination with HT and single HT was capable to display increased glutathione oxidation. Interestingly, despite HT triggering a more favorable antioxidant response, these effects were not associated with better CO2 assimilation (A) and photosystem II activity (FPSII). Cashew plants under WD and HT conditions displayed a significant decrease in A and FPSII. Collectively, our data suggest that cashew plants are not able to display effective antioxidant protection in response to a simultaneous condition of WD and HT. These finds indicate that systemic interaction among the various abiotic factors existing in a semiarid environment such as water deficit, heat, salinity, and high light is crucial to determining the performance of cashew plants under field conditions.