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WAGNER ROMULO LIMA LOPES FILHO

AJUSTES FISIOLÓGICOS E BIOQUÍMICOS EM PLANTAS DE PALMA DE ÓLEO (*Elaeis guineensis* Jacq.) SUBMETIDAS A EVENTOS REPETITIVOS DE DÉFICIT HÍDRICO

BELÉM – PA
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Tese apresentada à Universidade Federal Rural da Amazônia, como parte das exigências do Programa de Pós-Graduação em Agronomia, para obtenção do título de Doutor.

Área de concentração: Produção vegetal em sistemas agrícolas

Orientador: Prof. Dr. Hugo Alves Pinheiro

Co-orientador: Dr. Roberto Lisboa Cunha

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SUMÁRIO

RESUMO

O cultivo de palma de óleo (*Elaeis guineensis* Jacq.) estão sujeitos a períodos repetitivos de déficit hídrico e reidratação devido aos padrões de distribuição e frequência das chuvas que ocorrem na região Amazônica. Em algumas espécies, os eventos repetitivos de déficit hídrico disparam respostas fisiológicas e bioquímicas que culminam na aclimatação da maquinaria fotossintética e melhoram as respostas das plantas ao estresse. Este tipo de ajuste é conhecido como aclimatação ao estresse e ainda não foi avaliado em palma de óleo. Com isso, este trabalho objetivou avaliar a capacidade de aclimatação da maquinaria fotossintética e do sistema antioxidante da palma de óleo em função de déficit hídrico repetitivo. Para isso, as respostas fisiológicas de plantas de palma de óleo submetidas a um (WD 1x), dois (WD 2x) e três (WD 3x) eventos de déficit hídrico foram comparadas ao comportamento de plantas mantidas sob plena irrigação (controle). Os eventos de déficit hídrico foram conduzidos pelo tempo necessário para que o potencial hídrico na antemanhã (Ψ_{pd}) das plantas estressadas atingisse aproximadamente –2,5 MPa. De acordo com cada tratamento, a irrigação das plantas estressadas era retomada para induzir a completa recuperação das plantas antes que um novo evento de déficit hídrico fosse aplicado. As avaliações dos tratamentos foram iniciadas no momento da indução do terceiro, do segundo e do primeiro evento de estresse para os tratamentos WD 3x, WD 2x e WD 1x, respectivamente. Foi observada uma rápida diminuição do Ψ_{pd} nos primeiros sete dias de estresse, com médias de –1,81 MPa nas plantas WD 1x e –1,47 MPa nas WD 2x e WD 3x. A desidratação das plantas por sete dias levou a quedas acentuadas na taxa de assimilação líquida do CO_2 (*A*), porém, médias menores nas plantas WD 1x que nas plantas WD 3x. A condutância mesofílica ao CO_2 foi menor nas plantas WD 1x até o dia 21. A concentração de CO_2 no cloroplasto diminuiu igualmente nas plantas WD 1x e WD 3x até o dia 14, sendo menor nas WD 1x nos dias subsequentes. As plantas WD 1x apresentaram menor atividade da Ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco), menor taxa de regeneração da Ribulose 1,5 bisphosphate (J_{max}) e maiores taxas de fotorrespiração e respiração que os outros tratamentos. Além disso, as plantas WD 1x apresentaram menores valores médios de máxima eficiência quântica do fotossistema II (PSII) e máxima eficiência quântica do PSII em dada intensidade e isto ocorreu paralelamente a uma maior concentração de aldeído malônico (MDA). As diminuições na *A* das plantas estressadas foram acompanhadas por aumentos na razão entre concentração de CO_2 subestomática e ambiente (C_i/C_a) e redução significativa na eficiência de carboxilação (E_i/C) nas plantas WD 1x em relação às plantas WD 3x. A F_v/F_m nas plantas avaliadas aos 28 dias de avaliação não diferiu entre plantas controle e WD 3x, mas foi 22,8 % menor nas plantas WD 1x em relação ao controle. Foram observadas ainda expressivas reduções em ETR, ligado a aumentos no NPQ em plantas WD 1x. Em relação às plantas controle, o ETR/*A* foi aumentado 426,5% e 200% em plantas WD 1x e WD 3x. A atividade da SOD e GR aumentaram 124 % e 270% em plantas WD 3x, enquanto 68,7% e 30% em plantas WD 1x. Concentrações de H_2O_2 e MDA aumentaram 127% e 110% em plantas WD 1x, sem qualquer efeito no H_2O_2 e MDA em WD 3x em relação a controle. Assim, concluímos que a palma de óleo sofreu fortes diminuições na *A* mediadas por fatores estomáticos e não estomáticos, pois são capazes de aclimatar seu maquinário fotossintético, assim como modular seu sistema antioxidante melhorando seu desempenho quando submetidas a eventos repetitivos de déficit hídrico.

Palavras-chave: Aclimatação fotossintética, danos oxidativos, sistemas antioxidantes, secas sucessivas

ABSTRACT

Oil palm (*Elaeis guineensis* Jacq.) plantations are prone to repetitive periods of water deficit and re-hydration due to distribution patterns and rains in the region. In some species, repetitive water deficit events trigger physiological and biochemical responses that culminate in the acclimating of photosynthetic machinery and improve plant responses to stress. This adjustment is known as stress acclimation, and has not yet been evaluated in oil palm. With that, this work aimed to evaluate the ability of oil palm in acclimating their photosynthetic machinery and antioxidant system in response of repetitive water deficit events. For this, the physiological responses of oil palm plants submitted to one (WD 1x), two (WD 2x) and three (WD 3x) water deficit events were compared to well-watered plants (control). Water deficit events were conducted until the predawn leaf water potential (Ψ_{pd}) of the stressed plants reached around –2.5 MPa. According to each treatment, the irrigation of stressed plants was resumed to induce the complete recovery of plant water status before a new water deficit event was applied. Treatment evaluations were performed during the induction of the third, second, and first stress events for WD 3x, WD 2x, and WD 1x treatments, respectively. A fast decrease in Ψ_{pd} was observed in the first seven days of stress, with averages of –1.81 MPa in WD 1x plants and –1.47 MPa in WD 2x and WD 3x plants. Dehydration of plants for seven days led to sharp decreases in the net CO_2 assimilation rate (*A*), however, averages in WD 1x plants were lower than in WD 3x plants. The mesophyll conductance to CO_2 was lower in WD 1x plants until day 21. The chloroplast concentration of CO_2 decreased equally in WD 1x and WD 3x plants until day 14, being lower in WD 1x on subsequent days. The concentration of CO2 in the chloroplast decreased equally in WD 1x and WD 3x plants until day 14, being lower in WD 1x on subsequent days. WD 1x plants showed lower activity of ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco) and lower regeneration rate of Ribulose 1,5 bisphosphate (J_{max}) in parallel to higher rates of photorespiration and respiration than the other treatments. In addition, WD 1x plants showed lower average values of maximum quantum efficiency of photosystem II (PSII) (F_v/F_m) and maximum quantum efficiency of PSII at a given light intensity and this occurred in parallel with a higher concentration of malondialdehyde (MDA). Decreases in *A* of stressed plants were accompanied by increases in the substomatal to ambient CO_2 ratio and significant reduction in carboxylation efficiency in WD 1x plants compared to WD 3x plants. F_v/F_m in plants evaluated at 28 days of evaluation did not differ between control and WD 3x plants, but it was 22.8% lower in WD 1x plants compared to the control. Significant reductions in ETR were also observed, linked to increases in NPQ in WD 1x plants. In relation to the control plants, the ETR/A was increased by 426.5% and 200% in WD 1x and WD 3x plants. The activity of SOD and GR increased by 124% and 270% in WD 3x plants, while 68.7% and 30% in WD 1x plants. H_2O_2 and MDA concentrations increased by 127% and 110% in WD 1x plants, with no effect on H_2O_2 and MDA in WD 3x relative to control. Thus, we conclude that oil palm suffered strong decreases in *A* mediated by stomatal and non-stomatal factors, as they were able to acclimatize their photosynthetic machinery, as well as modulate their antioxidant system and improve their physiological performance when subjected to repetitive events of water deficit.

Keywords: Photosynthetic acclimation, Oxidative damages, antioxidant systems, successive droughts

1. CONTEXTUALIZAÇÃO

A palma de óleo (*Elaeis guineensis* Jacq.) é uma palmeira de origem africana que fornece uma das mais versáteis fontes de óleos vegetais do mundo (Alves et al., 2011). Seu fruto dá origem ao óleo de palma e palmiste, utilizados majoritariamente na indústria alimentícia, mas também empregado nas indústrias farmacêutica, cosmética e energética (Mba et al., 2015, ABRAPALMA, 2016).

O Brasil é o nono produtor mundial na produção de óleo de palma mundial. Em 2020, a produção brasileira de óleo foi de 395.000 ton (IBGE, 2020). O estado do Pará se destaca como maior produtor de frutos de palma de óleo, sendo responsável por cerca de 90% da produção brasileira, cuja área total cultivada em 2020 foi de 231.669 ha, sendo que aproximadamente 40 mil hectare advém da agricultura familiar (SEDAP, 2020). Os plantios de palma de óleo no Pará estão instalados na mesorregião do nordeste paraense, abrangendo, principalmente, os municípios do Acará, Moju, Tailândia, Concórdia do Pará e Tomé-Açu (SEDAP, 2020).

O cultivo de palma de óleo no Pará estão instalados em regiões com relativa abundância hídrica anual. Contudo, essas áreas estão sujeitas a ocorrência de curtos períodos de deficiência hídrica do solo decorrentes da redução temporal das chuvas no período mais seco do ano (de julho a novembro), situação que se agrava em anos de ocorrência de El Niño (Cai et al., 2014; Raza et al., 2019).

As reduções no crescimento vegetativo e na produção causadas pelo prolongamento do déficit hídrico são resultado de um processo que se inicia em alterações fisiológicas e bioquímicas que reduzem a produção de fotoassimilados (Méndez et al., 2012; Corley et al., 2018; Silva et al., 2017; Viana et al., 2019; Yehouessi et al., 2019; Yono et al., 2019). As principais alterações fisiológicas causadas pelo déficit hídrico em palma de óleo são as reduções nas taxas de *A*, condutância estomática (g_s) e transpiração (E), diminuição da atividade da Rubisco, degradação de pigmentos cloroplastídicos e indução de estresse oxidativo evidenciado pelo aumento de aldeído malónico (MDA), sendo a magnitude dessas reduções dependente da duração do estresse (Silva et al. 2016, 2017). Alterações nos parâmetros de fluorescência evidenciando a ocorrência de danos foto-oxidativos também têm sido relatados em plantas de palma de óleo submetidas ao déficit hídrico (Suresh et al. 2010, 2012).

Tem sido demonstrado em algumas espécies que exposições repetitivas ao déficit hídrico melhoram sua memória bioquimica para recuperar quando comparadas a indivíduos que passam pela primeira exposição ao estresse. Essa melhoria é evidenciada por aumentos no conteúdo relativo de água e nas taxas de assimilação líquida de CO_2 (Ding et al., 2012; Galle et al., 2011; Menezes-Silva et al., 2017; Nosalewicz et al., 2018). Além disso, fatores estomáticos e bioquímicos regulam diferencialmente a fotossíntese em uma resposta que varia de acordo com a espécie, conforme demonstrado em plantas de arroz, trigo e milho submetidas a um único evento de deficit hídrico (Perdomo et al., 2017).

Algumas espécies como *Craterostigma plantagineumto* (Liu et al., 2019); *Festuca arundinácea*, *Lolium perene* (Nosalewicz et al., 2018); *Coffea canephora* (Menezes-Silva et al., 2017); *Quercus ilex* e *Cistus albidus* (Galle et al., 2011); apresentam uma aclimatação fisiológica quando expostas a recorrentes períodos de déficit hídrico quando comparado com indivíduos que experimentam o estresse hídrico uma única vez. Tal aclimatação culminou em uma maior capacidade das plantas em suportar e se recuperar do estresse. Com a intensificação do estresse, a energia de excitação luminosa se torna superior à energia utilizada no processo fotossintético, inicialmente causando a redução na taxa de transporte de elétrons e posteriormente levando à saturação da cadeia transportadora de elétrons; provocando a formação de espécies reativas de oxigênio. Estas, por sua vez, promovem reações oxidativas que levam à danos estruturais no aparato fotossintético (Foyer et al., 2012). Essas alterações bioquímicas levam a uma redução na taxa de assimilação de CO_2 (Pilon et al., 2018, Lawrence et al., 2018).

Considerando-se o conhecimento prévio acerca das respostas fisiológicas da palma de óleo a um único evento de deficit hídrico, em que foi observada uma plasticidade de ajustes fisiológicos e ativação do sistema antioxidante (Silva et al., 2016) e considerando-se que as plantas são capazes de sobreviver a potenciais hídricos do solo muito baixos (–4,0 MPa, acessado por meio da medição do potencial hídrico foliar na ante-manhã; Silva et al. 2017), este trabalho foi desenvolvido a fim de testar as seguintes hipóteses: (i) a sensibilidade das plantas de palma de óleo a um ou mais eventos de deficit hídrico dá-se em função das fortes diminuições na *A* mediadas por fatores estomáticos e não estomáticos de regulação da fotossíntese, e (ii) plantas de palma de óleo são capazes de aclimatar sua maquinaria fotossintética e modular seu sistema antioxidante para lidar de forma mais adequada com o estresse oxidativo mediado pelos eventos repetitivos de deficit hídrico.

Do exposto, o objetivo geral deste trabalho foi investigar os ajustes fisiológicos e bioquímicos relacionados à regulação da fotossíntese em plantas de palma de óleo submetidas a eventos repetitivos de déficit hídrico. Como objetivos específicos, pretendeu-se (i) avaliar as limitações estomáticas e não estomáticas à fotossíntese e aclimatação da maquinaria fotossintética aos eventos de déficit hídrico repetitivo (Capítulo 1); e (ii) testar a capacidade de aclimatação do sistema antioxidante enzimático em plantas de palma de óleo submetidas a eventos repetitivos de déficit hídrico (Capítulo 2).

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2. CAPITULO 01. PHYSIOLOGICAL RESPONSES OF YOUNG OIL PALM (*Elaeis guineensis* Jacq.) PLANTS TO REPETITIVE WATER DEFICIT EVENTS*

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ABSTRACT

Stomatal and non-stomatal limitations to photosynthesis and acclimation of the photosynthetic machinery to repetitive water deficit (WD) events were investigated in oil palms (*Elaeis guineensis* Jacq.). For this, well-watered plants (control treatment) were compared with plants subjected to one, two, and three WD events imposed by withholding irrigation until their predawn leaf water potential (Ψ_{pd}) reached around −2.5 MPa. Treatment comparisons were performed over 28 days. The Ψpd decreased equally between WD treatments. Decreases in net CO_2 assimilation rate (*A*) were similar between stressed plants until day 7. In the following days, the *A* was higher in plants thrice stressed than in those stressed once. Stomatal conductance decreased similarly between WD treatments, but mesophyll conductance to CO_2 was lower in plants subjected to a single WD. Chloroplast CO_2 concentration decreased similarly between plants subjected to one and three WD events until day 14, but it was lower in the former on subsequent days. Plants subjected to a single WD event showed lower Ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco) activity, maximum rate of electron transport (J_{max}), and higher rates of photorespiration and dark respiration than other treatments. Plants subjected to one WD event also showed lower maximum quantum efficiency of photosystem II (PSII) photochemistry and PSII maximum efficiency concomitantly with higher malondialdehyde content. The results support that repetitive WD events induce the acclimation of the photosynthetic machinery in oil palm through adjustments in carboxylase Rubisco activity, J_{max}, photorespiration, and respiration rates, as well as attenuating oxidative damages to PSII and membrane lipids.

Keywords: A-C_i curves, Oxidative damages, Photorespiration, Photosynthetic acclimation, Recurring drought, Stress memory

Abbreviations: *A*, net CO_2 assimilation rate; C_c, chloroplast CO_2 concentration; C_i/C_a, substomatal to ambient CO_2 concentration ratio; *E*, transpiration; F_v/F_m, maximum quantum efficiency of PSII photochemistry; ETR, apparent electron transport rate; ETR_A, fraction of the total ETR used in CO_2 assimilation; ETR_P, fraction of the total ETR used in photorespiration; F'_v/F'_m, PSII maximum efficiency; g_m, mesophyll conductance to CO_2; g_s, stomatal conductance to water vapor; J_{max}, maximum rate of electron transport; MDA, malondialdehyde; NPQ, non-photochemical quenching; PSII, photosystem II; q_L, fraction of PSII centers that are open; q_P, photochemical quenching; R_D, dark respiration; R_P, photorespiratory rate of Rubisco; Rubisco, ribulose 1,5 bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5 bisphosphate; TPU, triose-P utilization rate; V_{cmax}, maximum velocity of Rubisco carboxylation; WUE, instantaneous water use efficiency; Ψ_{pd}, predawn leaf water potential.

2.1. Introduction

The oils extracted from oil palm (*Elaeis guineensis* Jacq., Arecaceae) fruits have physical and chemical properties that allow their use in several industrial processes, including the manufacturing of foods, cosmetics, medicines, lubricants, and biodiesel (Barcelos et al., 2015; Mba et al., 2015). The production of oil palm fruits and palm oil to meet industrial demands comes from extensive palm plantations predominantly cultivated as monoculture in tropical regions. The largest palm oil producers are Indonesia and Malaysia, which accounted for 58 and 26 %, respectively, of the total global production of palm oil in 2020 (USDA-FAS, 2021). In Brazil, the oil palm plantations are predominantly found in the northern region, particularly in the state of Pará, and in 2020, this state accounted for 84 % of the total Brazilian production of palm oil harvested from around 180,000 ha (USDA-FAS, 2021). Regardless of location, oil palm plantations are predominantly irrigated by rainfall (Woittiez et al., 2017). Thus, depending on the location, negative effects on fruit yield are observed when the annual water deficit is greater than 50–200 mm, with additional yield losses between 10 and 20 % for every 100 mm of water deficit from the threshold value inherent for each location (Woittiez et al., 2017). Therefore, water deficit is an important environmental stress factor that limits the production of oil palm fruits worldwide.

The sensitivity of oil palm to water deficit (WD) has been widely reported. In vegetative stages, such sensitivity is due, in part, to the fast and significant loss of turgor as stress develops (Bayona-Rodríguez et al., 2016; Silva et al., 2016, 2017; Najihah et al., 2019). With the loss of turgor, the vegetative growth of both aboveground organs and the root system is negatively affected (Rivera-Mendes et al., 2016; Silva et al., 2017; Najihah et al., 2019). In physiological terms, the progressive leaf dehydration leads to decreases in the net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s), and transpiration (E) in parallel to unchanged or to increased substomatal CO_2 concentration (C_i) (Bayona-Rodríguez et al., 2016; Silva et al., 2016, 2017; Bayona-Rodriguez and Romero, 2019; Viana et al., 2019) and induces increases in the activities of glycolate oxidase (GLOx) and catalase (CAT), suggesting greater photorespiration in stressed plants (Silva et al., 2016). Changes in chlorophyll fluorescence parameters in WD-stressed oil palm plants indicate that this species is sensitive to photo-oxidative damages, which may arise from an imbalance between the photochemical and biochemical reactions of photosynthesis (Suresh et al., 2010; Bayona-Rodríguez et al., 2016). In the reproductive stage, the decrease in oil palm fruit production with soil water shortage can be evidenced by the reductions in both the number and fresh mass of bunches (Viana et al., 2019). As reviewed by Corley and Tinker (2016), the decreases in oil palm fruit production are related to the WD effects in inducing delays in floral initiation and inducing increases in the male/female ratio and the rate of flower abortion.

Much of the current knowledge about oil palm responses to WD has been derived from experiments in which plants are subjected to a single stress event. Thus, comparisons between irrigated and stressed plants have been carried out under predefined soil water potentials or throughout stress development (Suresh et al., 2010; Rivera-Mendes et al., 2016; Silva et al., 2016, 2017; Viana et al., 2019). However, experiments coupling A-Ci curves and chlorophyll fluorescence data to provide a better understanding of how stomatal issues and photorespiration may impair net photosynthesis are incipient in WD-stressed plants of oil palm (Silva et al., 2015; Pilon et al., 2018). On the other hand, the oil palm responses to repeated WD events interspersed with complete rehydration of plants remain understudied. This approach is of great significance because in non-irrigated plantations, the soil water replacement depends exclusively on rainfall. Thus, in areas subject to well-defined dry seasons throughout the year, such as northern Brazil, repetitive WD events can be observed between successive rains during the dry season.

In *Nicotiana sylvestris* and *Coffea canephora*, the repetitive events of WD and rehydration trigger physiological, biochemical, and molecular responses that culminate in adjustments in the photosynthetic apparatus and net photosynthesis, in photorespiration and respiration rates (Galle et al., 2009; Menezes-Silva et al., 2017). Such adjustments would allow plants subjected to a previous WD event to exhibit better physiological responses when submitted to subsequent periods of stress. This ability to memorize stress would involve epigenetic mechanisms, such as changes in chromatin condensation and DNA methylation (He and Li, 2018).

Taking our prior findings in oil palm physiology under drought conditions (Silva et al., 2016, 2017), we hypothesized that (i) the sensitivity of oil palm plants to single and repetitive WD conditions is due to the strong decreases in *A* mediated by both stomatal and non-stomatal traits limiting photosynthesis, and (ii) oil palms can acclimate their photosynthetic machinery to properly cope with repetitive WD events. To test these hypotheses, we grew oil palm plants in large containers and induced single and repetitive WD events. Then, we compared the stressed plants with irrigated counterparts at different time points by coupling leaf gas exchange (including A-Ci curves) and chlorophyll fluorescence measurements, thus aiming to (i) provide a better understanding of stomatal and non-stomatal factors impairing *A* in response to a single WD event, and (ii) evaluate the ability of oil palm plants in adjusting their photosynthetic machinery and photorespiration to attenuate photo-oxidative damages to PSII and membrane lipids in response to repetitive WD events.

2.2. Materials and methods

2.2.1. Plant material and growth conditions

The work was carried out from October 15 to December 15, 2019. Four-month-old oil palm seedlings (Elaeis guineensis Jacq., Arecaceae, Tenera cultivar), produced in 20-L polyethylene bags, were arranged in a seedling nursery at the Institute of Agricultural Sciences (ICA), Federal Rural University of the Amazon (UFRA), in Belém, state of Pará, Brazil (01°27′19″ S and 48°26′19″ W). After acclimation for 60 days, the seedlings were transferred to 50-L containers (49 × 59 cm, height × diameter) filled with a mixture of Dystrophic Yellow Latosol and poultry litter (3:1, v/v). The substrate acidity was corrected using 30 g of dolomitic limestone (Plantei Garden Center Ltda ME, Salto Grande, Brazil) per pot, which was applied before transplanting the seedlings. Every 30 days after transplanting, the plants were fertilized using 10 g 13-11-21 NPK (YaraMila PALMAE, Yara Brazil S.A., Porto Alegre, Brazil), 2.5 g magnesium sulfate (YaraTera KRISTA MgS, Yara Brazil S.A., Porto Alegre, Brazil) and 1 g boron + calcium (YaraVita CaBtrac, Yara Brazil S.A., Porto Alegre, Brazil) per pot. In addition, foliar fertilizations with 20 g L^{-1} 2020-20 NPK + micronutrients (Plant-Prod 20-20-20 Classic, Master Plant-Prod Inc., Brampton, Canada) were performed at 15-day intervals before induction of WD treatments. The irrigation of the plants was performed daily, at 8:00 h (first irrigation) and 17:00 h (second irrigation), aiming to keep the soil as close to field capacity as possible. The volume of water applied in each treatment was estimated based on the variation in substrate moisture between irrigations. Therefore, substrate moisture was often determined as described by Klar et al. (1966).

2.2.2. Treatments and experimental design

Plants were cultivated for 17 months under daily irrigation before the experimental setup (i.e., cultivation period of plants for 510 days, Fig. 1). Then, 20 plants with similar aboveground morphology (2.5 ± 0.2 m tall, 65 ± 1.5 cm in the circumference of the stem, 25 ± 1 open leaves, and 3 ± 1 arrow leaves) were selected and randomly distributed among four treatments: Control, in which the plants were irrigated daily throughout the experimental period and three WD treatments, in which the plants were respectively subjected to one (WD 1x), two (WD 2x), or three (WD 3x) events of stress.

Treatment induction was performed in three rounds as denoted in Fig. 1. In round 1, the plants of the control, WD 1x, and WD 2x treatments were irrigated daily to field capacity, while the plants of the WD 3x treatment were subjected to the first WD event. In round 2, the plants of the control and WD 1x treatments were irrigated daily at field capacity, while the plants of the WD 2x and WD 3x treatments were respectively subjected to their first and second WD events. In rounds 1 and 2, the average of Ψ_{pd} for plants maintained under full irrigation was around −0.07 ± 0.02 MPa, while the WD events extended until the Ψ_{pd} of the stressed plants reached about −2.5 ± 0.02 MPa (21 days, Fig. 1). Then, the stressed plants were re-watered daily until the Ψ_{pd} and A did not differ from control plants (recovery period of 10 days, Fig. 1). After reaching full recovery, the plants were irrigated daily to field capacity for seven days before starting new WD event (lag period, Fig. 1).

The treatment evaluation period was performed throughout round 3 (Fig. 1). For this, the irrigation was kept for control plants and completely withheld for WD treatments. This way, control plants remained unstressed ($\Psi_{pd} = -0.07 \pm 0.02$ MPa) and plants from the WD 1x, WD 2x, and WD 3x treatments were respectively subjected to their first, second, and third WD events. Physiological measurements and sampling for biochemical assays were performed at the moment of watering regime differentiation (named day 1 in the figures) and repeated every seven days until that the Ψ_{pd} for plants of at least one of WD treatment reached around -2.5 ± 0.02 MPa. The evaluation period lasted 28 days (Fig. 1).

Considering that A-C_i curves are time-consuming work and considering the number of plants to be accessed, we set up the experiment in a randomized block design, in a split-plot scheme, with the watering regimes (WR) in the plots and evaluation times

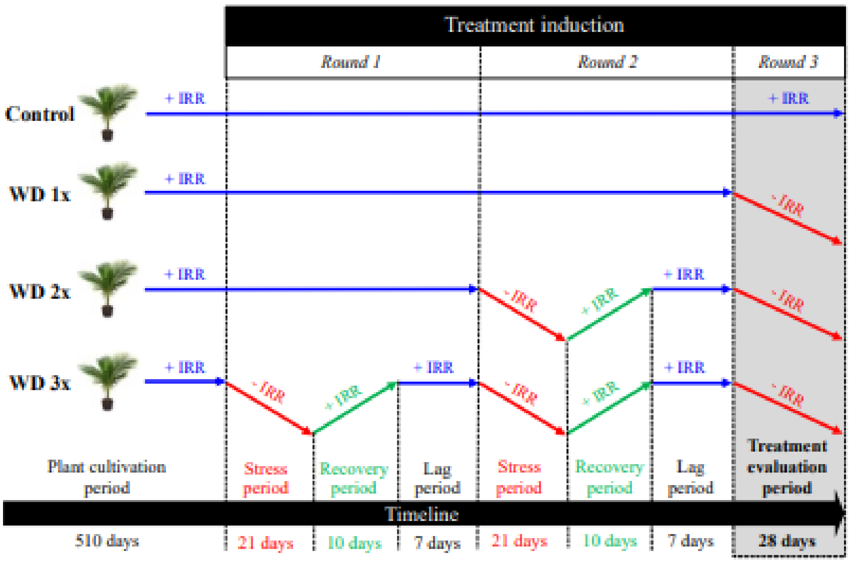

Fig. 1

Fig. 1. Scheme of the experimental setup and treatment evaluation. Treatments were induced in three rounds (Rounds 1, 2, and 3), in which well-watered conditions were provided by irrigating the plants daily (+ IRR) and water deficit (WD) events were imposed by withholding irrigation (− IRR). At the end of each stress period in rounds 1 and 2, the irrigation was resumed to induce the physiological recovery of the plants. After that, plants were irrigated daily for seven days (lag period) before starting a new WD event. The control treatment consisted of fully irrigated plants, while stress treatments consisted of plants subjected to one, two, and three WD events (i.e., WD 1x, WD 2x, and WD 3x treatments). Treatment evaluation was performed throughout round 3 (for details see Materials and methods).

(T) in the subplots. Five blocks were set up and each block was composed of one plant per treatment (Control, WD 1x, WD 2x, and WD 3x).

Considering that A-C_i curves are time-consuming work and considering the number of plants to be accessed, we set up the experiment in a randomized block design, in a split-plot scheme, with the watering regimes (WR) in the plots and evaluation times (T) in the subplots. Five blocks were set up and each block was composed of one plant per treatment (Control, WD 1x, WD 2x, and WD 3x).

2.2.3. Climate conditions during treatment evaluation

The data for air temperature (T_{air}) and relative air humidity (RH) outside the nursery were collected daily from days 1–28 between 6:00 and 18:00 h in 1-h intervals. The data for each day were the average of 13 records ± SE (Fig S1A). The T_{air} and RH data from inside the nursery were collected during physiological measurements (i.e., at days 1, 7, 14, 21, and 28), between 8:30 and 10:30 h, in 30-min intervals, using a thermohygrometer placed inside the nursery. Photosynthetically active radiation (PAR) inside the nursery was recorded with a quantum sensor attached to the gas analyzer chamber. Climate data from inside the nursery are the average of four records ± SE (Fig S1B). The vapor pressure deficit (VPD) was calculated as described by Alvarenga et al. (2014). Although diurnal averages of the T_{air}, RH, and VPD outside nursery varied between days 1 and 28 (Fig. S1A), the T_{air}, RH, VPD, and PAR values from inside the nursery were almost constant during physiological measurements. Thus, the averages of T_{air}, RH, VPD, and PAR for the evaluation period were 32.2 ± 0.2 °C, 67.3 ± 0.4%, 1.63 ± 0.02 kPa, and 672 ± 32 μmol photons m^{-2} s^{-1}, respectively (Fig. S1B).

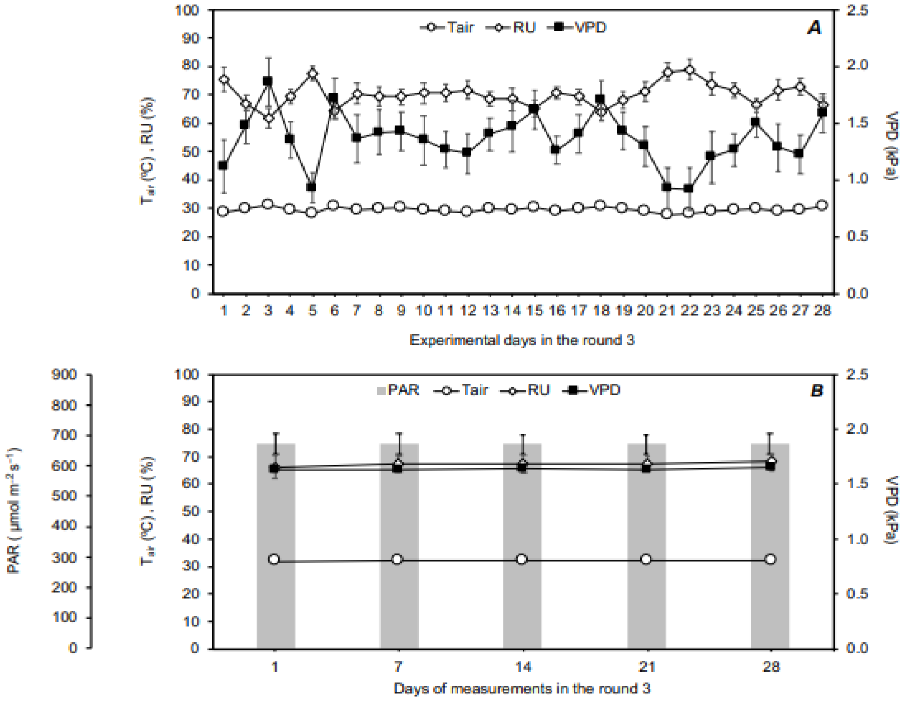

Fig. S1. Diurnal climate conditions recorded outside nursery throughout round 3 (A) and climate conditions recorded inside nursery throughout physiological measurements (B). T_{air}, is the air temperature; RU is the relative air humidity; VPD, is the vapor pressure deficit (VPD); and PAR is the photosynthetically active radiation (PAR). Data for each time point in figures A and B are the mean ± SE (for details see Materials and methods).

2.2.4. Plant water status

One leaflet per plant was sampled from the median portion of the third leaf counted from the apices and immediately enclosed in a Scholander-type pressure chamber (m670, Pms Instrument Co., Albany, USA) to determine the pre-dawn leaf water potential (Ψ_{pd}). Measurements were carried out between 4:00 and 5:00 h.

2.2.5. *A*-C_i curves and leaf gas exchange measurements

The *A*-C_i curves were performed as described by Pilon et al. (2018), with some modifications. The infrared gas analyzer (LI-6400 XT; LI−COR Biosci. Inc., Nebraska, USA) was adjusted to work under constant PAR of 1100 μmol photons m^{-2} s^{-1} (Suresh et al., 2010) and at the following CO_2 concentrations: 400, 300, 200, 100, 50, 400, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1500 and 1800 μmol mol^{-1}. Data for each CO_2 concentration were recorded within a maximum wait time of 120 s. The maximum velocity of Rubisco carboxylation (V_{cmax}), maximum electron transport rate (J_{max}), and dark respiration (R_D) were estimated from A-C_i curves and chlorophyll fluorescence measurements according to Ethier and Livingston (2004). Apparent electron transport rate (ETR, estimated Maxwell and Johnson, 2000) was divided in the fraction of the total ETR used in CO_2 assimilation [$ETR_A = 1/3(ETR + 8 \times (A + R_D))$] and in the fraction used in photorespiration [$ETR_P = 2/3(ETR - 4 \times (A + R_D))$] (Galmés et al., 2007). Chloroplast CO_2 concentration (C_c) was calculated according to Ennahli and Earl (2005) as $C_c = (ETR_A/ETR_P) \times O/K_s$, in which O is the O_2 concentration at the carboxylation site of Rubisco (0.21) and K_s (4.51) represent the CO_2/O_2 specificity of Rubisco. Mesophyll conductance to CO_2 (g_m) was calculated as $g_m = A/(C_i - C_c)$, according to Galmés et al. (2007). The photorespiratory rate of Rubisco (R_P) was calculated according to Valentini et al. (1995) as $R_P = 1/12[ETR - 4(A + R_L)]$, in which R_L is the respiration rate under light conditions estimated as $R_L = 0.5 \times R_D$ (Niinemets et al., 2009). The triose-P utilization rate (TPU) was estimated as $TPU = (A + R_D)/3$ (Sharkey et al., 2007).

Leaf gas exchange (A, g_s, E, and substomatal to ambient CO_2 concentration ratio; C_i/C_a) and instantaneous water use efficiency ($WUE = A/E$) under ambient CO_2 concentration were obtained from the A-C_i curves, considering the average of the two measurements recorded at 400 μmol CO_2 mol^{-1} during the [CO_2] increment phase of the A-C_i protocol.

2.2.6. Chlorophyll fluorescence

Chlorophyll fluorescence measurements were performed using a fluorometer (LI-6400-40; LI-COR Biosci. Inc., Nebraska, USA). The sampled leaflets were dark-adapted for 30 min and illuminated with modulating light pulses to determine the initial fluorescence (F_0) and with a saturating light pulse to determine the maximum fluorescence (F_m). After that, a continuous actinic light was applied to reach the steady-state fluorescence (F_s), and a saturating white light pulse and a far-red light pulse were respectively applied to determine the maximum fluorescence (F'_m) and light-adapted F_0 (i.e., F'_0). The intensities and durations of the light pulses applied in these measurements exactly followed the methodology described by Lima et al. (2002). The maximum quantum efficiency of PSII photochemistry (F_v/F_m), PSII maximum efficiency (F'_v/F'_m), and non-photochemical quenching (NPQ) were estimated through equations described in Krause and Weis (1991). The fraction of PSII centers that are open (q_L) was estimated as $q_L = q_P$ (F'_0/F_s) (Kramer et al., 2004).

2.2.7. Biochemical assays

Leaf samples (100 mg FW) were collected from the middle portion of the third leaf expanded from the apex and immediately frozen in liquid nitrogen. The samples were kept at −80 °C until assays.

Rubisco (Ribulose 1,5 bisphosphate carboxylase/oxygenase, EC 4.1.1.39) was extracted by grinding leaf samples (100 mg FW) in 0.8 mL of extraction buffer consisting of 500 mM HEPES (pH 7.5), 2.5 % (w/v) and BSA, 10 mM benzamide, 100 mM $MgCl_2$, 10 mM ε-aminocaproic acid, 10 mM EDTA, and 10 mM EGTA (pH 8.0) (Geigenberger and Stitt, 1993). The slurry was centrifuged at 3,000 × g for 5 min at 4 °C, and the supernatant was collected for protein quantification (Bradford, 1976) and biochemical assays for the determination of Rubisco initial and final activities as well as the Rubisco activation state (Sulpice et al., 2007).

Lipid peroxidation was estimated by quantifying the concentration of malondialdehyde (MDA) according to Cakmak and Horst (1991) with minor modifications as described in Silva et al. (2016).

2.2.8. Statistical analysis

Data normality was tested by the Shapiro-Wilk test and equality of variances was tested by Levene's test. After that, data were submitted to ANOVA considering the split-plot design, with the watering regimes (WR) in the plots and the evaluation times (T) in the subplots. Differences between means were compared by Duncan's test ($P \leq 0.05$). Data in the figures refer to the means of five replicates ± standard error (SE). All statistical procedures were performed using R (v. 3.4.1; R Core Team, 2016).

2.3. Results

2.3.1. Leaf water potential, leaf gas exchange and chlorophyll fluorescence

The F values for Ψ_{pd}, leaf gas exchange, and chlorophyll fluorescence were significant for WR, T, and WR × T effects (Table 1). The stressed plants of each individual WD treatment showed a similar tendency to dehydration throughout the evaluation period, while Ψ_{pd} in control plants did not differ significantly over different time points (Fig. 2A). The most pronounced decreases in the Ψ_{pd} of the stressed plants were recorded between days 1 and 7 (Fig. 2A). During this first week of water shortage,

Table 1. F statistics for the effect of watering regimes (WR), time of evaluation (T), and their interaction (WR × T) on physiological and biochemical variables measured in young oil palm plants.

Variables	WR	T	WR x T
Ψ_{pd}	3328.5**	1842**	209.4**
A	7102.70**	6039.70**	548.60**
g_s	2286.40**	468.40**	119.10**
E	4089.70**	520.90**	65.60**
C_i/C_a	143.19**	164.41**	13.73**
WUE	91.05**	37.92**	10.67**
F_v/F_m	35.67**	12.05**	4.65**
F'_v/F'_m	263.84**	52.01**	10.78**
NPQ	262.87**	115.76**	14.58**
q_L	2543.97**	877.94**	104.13**
$\mathrm{V_{cmax}}$	1230.48**	2.89*	6.95**
$\mathrm{J_{max}}$	1798.51**	33.69**	14.12**
g_m	139.81**	28.97**	7.58**
C_c	3261.50**	633.90**	157.3**
$\mathrm{R_D}$	1981.29**	94.58**	70.69**
$\mathrm{R_P}$	2785.91**	312.71**	260.58**
$\mathrm{ETR_A}$	4116.70**	98.40**	11.10**
$\mathrm{ETR_P}$	87.34**	10.80**	26.01**
$\mathrm{ETR_A/ETR_P}$	1429.10**	14.08**	24.93**
Rubisco – FA	79.14**	10.66**	4.66**
Rubisco – AS	48.97**	11.54**	7.96**
TPU	79.86**	9.27**	7.85**
MDA	180.24**	24.06**	18.23**

*significant at $P < 0.05$, ** significant at $P < 0.01$

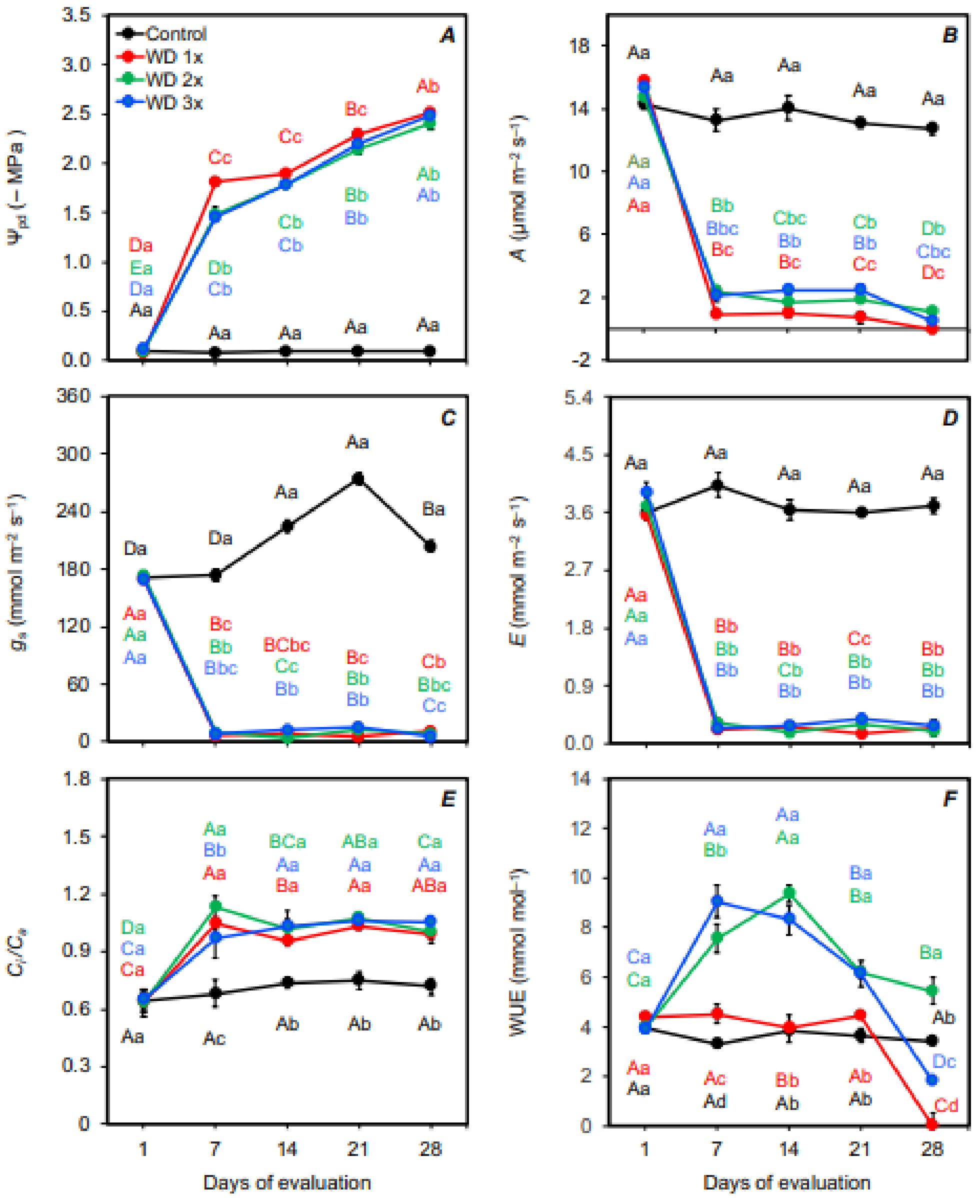

Fig. 2

Fig. 2. Time course of changes in pre-dawn leaf water potential **(A)**, net CO_2 assimilation rate **(B)**; stomatal conductance to water vapor **(C)**; transpiration rate **(D)**, substomatal to ambient CO_2 concentration ratio **(E)**, and instantaneous water use efficiency **(F)** in young oil palm plants subjected to well-watered conditions (control) and one (WD 1x), two (WD 2x), and three (WD 3x) water deficit events. Data are the means ± SE (n = 5). Different capital letters denote significant differences between averages for the same treatment measured on different experimental days, and different small letters denote significant differences between different treatments compared on the same experimental day (Duncan's test, $P \leq 0.05$).

the Ψ_{pd} decreased from –0.07 MPa to –1.81 MPa in WD 1x plants and from –0.11 MPa to –1.47 MPa in WD 2x and WD 3x plants. Further decreases in Ψ_{pd} were observed on subsequent days 14 and 21, with the lowest Ψ_{pd} averages recorded for WD 1x plants (Fig. 2A). However, differences between treatments were unobserved on day 28, when Ψ_{pd} reached the same mean value for all WD treatments (–2.47 MPa, Fig. 2A). These results suggest that the initial loss of turgor (up to day 21) was slightly faster in plants that had not yet been stressed (WD 1x) than in those previously subjected to one or two WD events. At least in part, the faster dehydration in WD 1x relative to WD 2x and WD 3x, particularly at day 7, may be explained by the higher number of leaves in WD 1x plants at the beginning of round 3 (Fig. S2), favoring higher whole-plant transpiration. Anyhow, such morphological differences did not exert significant effects in Ψ_{pd} between WD treatments accessed under more prolonged drought (day 28, Fig. 2A).

The dehydration of plants for seven days led to sharp decreases in A, $g_{s,}$ and E for all WD plants (Fig. 2B, C, and D). Regardless of the WD treatment, variations in A, $g_{s,}$ and E were almost negligible between days 7 and 28 (Fig. 2B, C, and D). The averages of A in WD 1x, WD 2x and WD 3x plants for the period between days 7 and 28 were, respectively, 0.64 μmol m^{-2} s^{-1}, 1.74 μmol m^{-2} s^{-1}, and 1.85 μmol m^{-2} s^{-1}, representing 95%, 86%, and 87% decreases relative to control plants (Fig. 2B). For the same period of stress, the averages of g_s were 7.67 mmol m^{-2} s^{-1} for WD 1x, 8.96 mmol m^{-2} s^{-1}for WD 2x, and 10.16 mmol m^{-2} s^{-1}for WD 3x, accounting for decreases around 96% relative to their control counterparts (Fig. 2C). Relative to the control, the stressed plants from all WD treatments showed similar decreases in E in all time points (Fig. 2D), whereas C_i/C_a in stressed plants of all WD treatments was around 1.4 times higher than in control plants accessed from day 7 onwards (Fig. 2E).

The plants exposed to a single WD event showed a 37% increase in WUE on day 7, but differences between treatments were unobserved on days 14 and 21. Furthermore, the WUE in WD 1x plants reached the lower mean value on day 28 (Fig. 2F). Relative to the control, the WD 2x and WD 3x plants, respectively, showed 129% and 174% increases in WUE on day 7 and 116% and 129% increases in WUE on day 14 (Fig. 2F). On day 21, WUE in WD 2x and WD 3x plants decreased relative to previous time points; however, the mean WUE for both treatments remained 70% greater than in control plants (Fig. 2F). Under more prolonged drought (day 28), the WUE in WD 2x and WD 3x plants were 59% higher and 47% lower relative to their control counterparts (Fig. 2F).

Relative to the control, the oil palm plants subjected to WD for the first time (WD 1x) showed significant decreases in F_v/F_m (18%) and F'_v/F'_m (43%) from day 7 (Fig. 3A and B). The means of F_v/F_m were similar between control, WD 2x, and WD 3x plants in

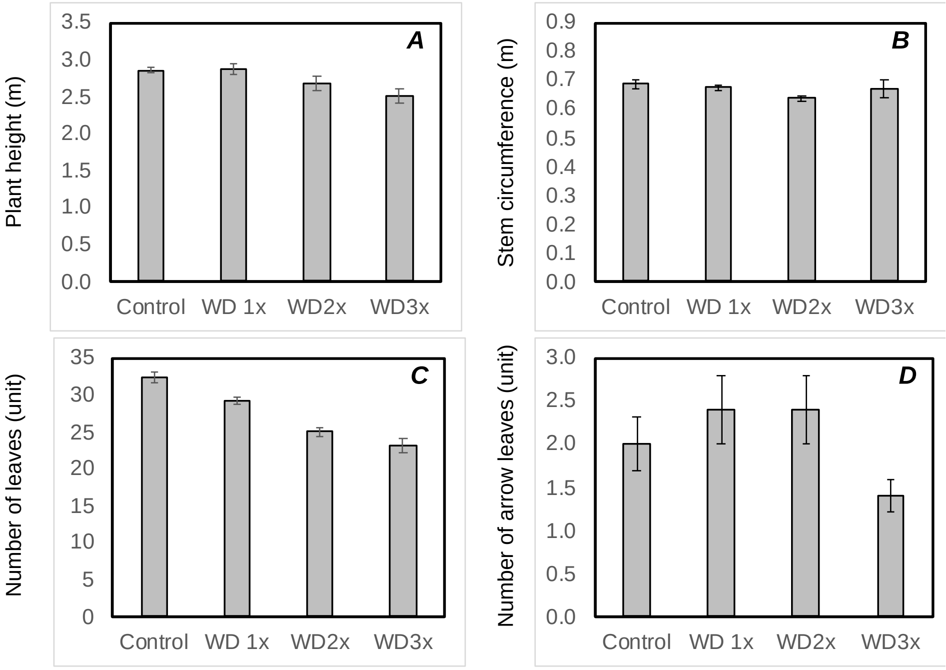

Fig. S2. Plant height (A), stem circumference (B), number of leaves (C), and number of arrow leaves (D) in oil palm plants from the different experimental treatments measured just prior to the round 3 onset. Data are the means ± SE (*n* = 5).

all evaluation times; however, the F'_v/F'_m was slightly reduced on day 7 (8% in WD 2x and 5% in WD 3x) and more significantly reduced between days 14 and 28 (42% in WD 2x and 18% in WD 3x), as shown in Fig. 3A and B. On days 7 and 14, q_L was decreased by 88% in WD 1x and by 66% in both WD 2x and WD 3x plants relative to respective control plants, while on days 21 and 28, the q_L reached values around zero in all WD treatments (Fig. 3C). The NPQ in stressed plants was higher than in plants kept under full irrigation at all time points (Fig. 3D). Thus, relative to the control, the NPQ increased by 173% in WD 1x plants and by 98% in both WD 2x and WD 3x plants. (Fig. 3D).

Given that the pattern of changes in leaf gas exchange and chlorophyll fluorescence between WD 2x and WD 3x plants was quite similar, we excluded WD 2x plants from treatment comparisons for *A*-*Ci* curves and biochemical data, and we also excluded day 1 because the differences between treatments were not significant. Compared to control plants, the averages of V_{cmax} and J_{max} on days 7 and 14 were more significantly reduced in WD 1x (83%) than in WD 3x (69%); however, on days 21 and 28, both variables were equally reduced by 85% in all WD treatments (Fig. 4A and B). Relative to the control, g_m progressively decreased in WD 1x and WD 3x plants (Fig. 4C). However, such decreases were more significant in WD 1x (58%) than in WD 3x plants (40%) (Fig. 4C). After 7 and 14 days under stress conditions, C_c in the WD treatments was decreased by about 68% compared to the control (Fig. 4D). On days 21 and 28, the magnitude of decreases in C_c differed between WD treatments, being more pronounced in WD 1x (40 %) than in WD 3x (20%) as shown in Fig. 4D. Seven days after withholding irrigation, the average of R_D in WD plants was 1.53 μmol m^{-2} s^{-1}, corresponding to a nine-time increase relative to control plants (Fig. 4E). With the stress progression, additional increases in R_D were observed only in the WD 1x plants, whose average on day 28 (2.50 μmol m^{-2} s^{-1}) was about 20 times greater than that in the control plants (Fig. 4E). The WD 1x plants showed higher R_P averages than the control plants, especially on day 14, when a 244% increase in the R_P of the stressed plants was observed (Fig. 4F). On the other hand, the mean R_P did not differ between control and WD 3x plants, regardless of evaluation time (Fig. 4F). Except on day 28, our data show that ETR_A was more reduced in WD 1x than in WD 3x (Fig. 4G). On the other hand, between days 7 and 21, the increases in ETR_P were more striking in WD 1x plants than

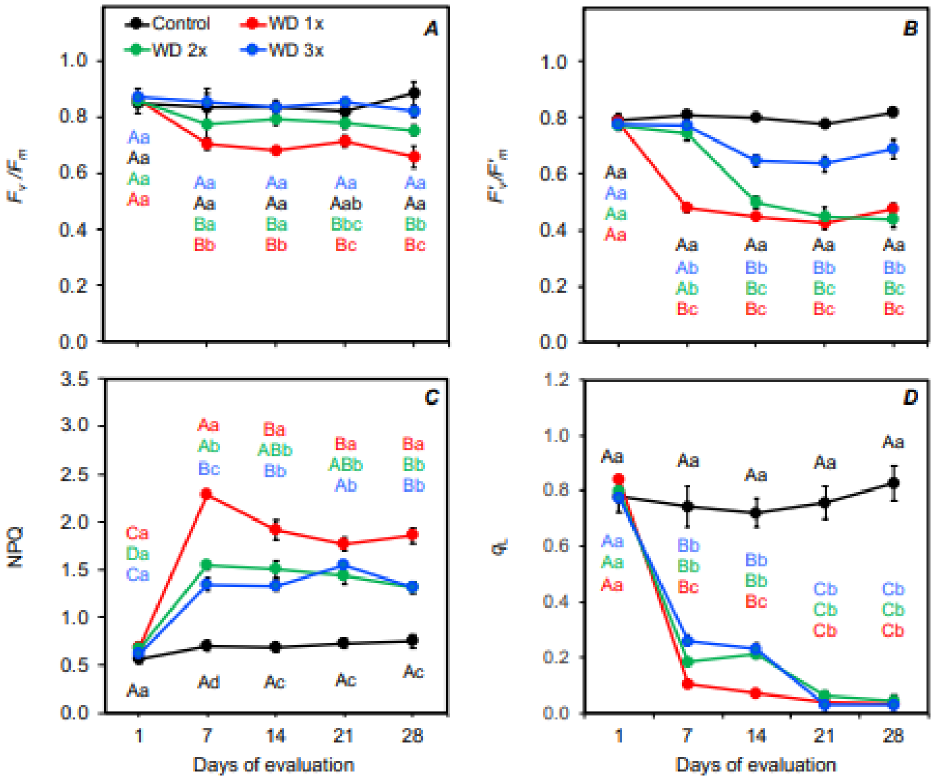

Fig. 3

Fig. 3. Time course of changes in the maximum quantum efficiency of PSII photochemistry **(A)**, PSII maximum efficiency **(B)**, fraction of PSII centers that are open **(C)**, and non-photochemical quenching **(D)** in young oil palm plants subjected to well-watered conditions (control) and one (WD 1x), two (WD 2x), and three (WD 3x) water deficit events. Data are the means ± SE (n = 5). Different capital letters denote significant differences between averages for the same treatment measured on different experimental days, and different small letters denote significant differences between different treatments compared on the same experimental day (Duncan's test, $P \leq 0.05$).

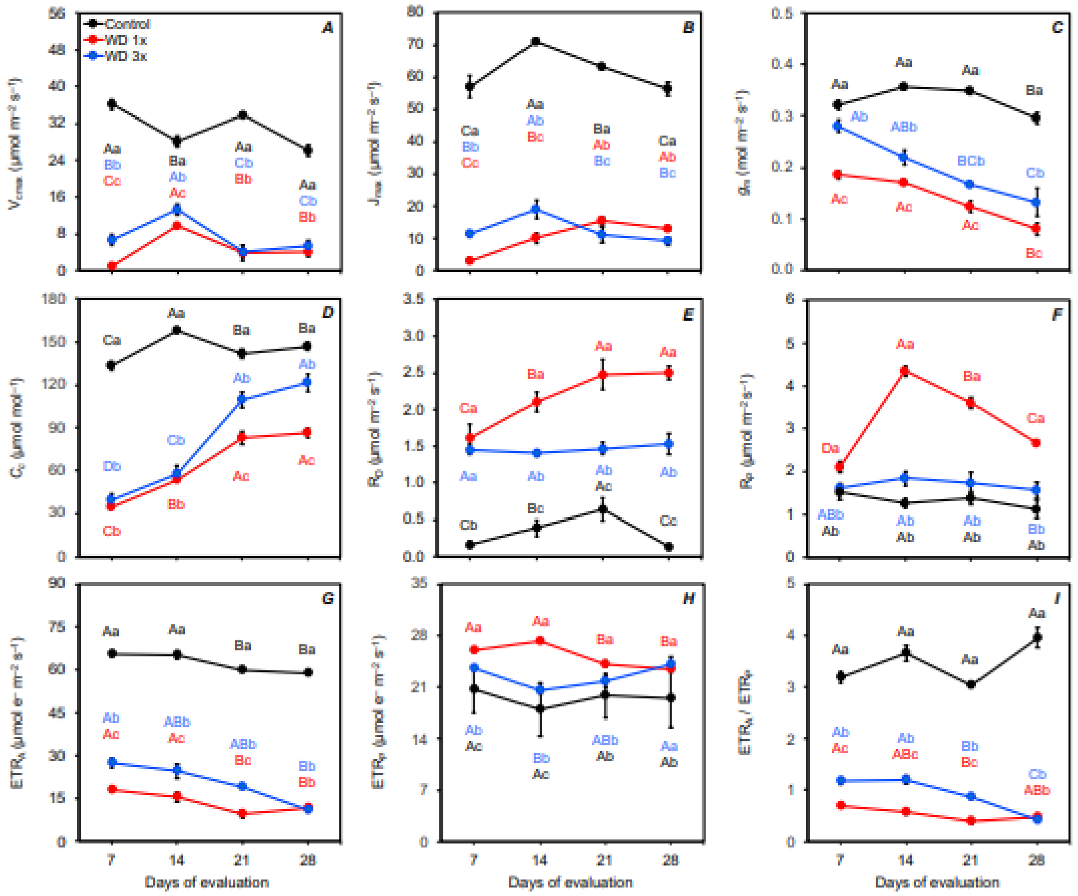

Fig. 4

Fig. 4. Time course of changes in the maximum velocity of Rubisco carboxylation **(A)**, maximum rate of electron transport **(B)**, mesophyll conductance to CO_2 **(C)**, chloroplast CO_2 concentration **(D)**, dark respiration **(E)**, photorespiratory rate of Rubisco **(F)**, fraction of the total ETR used in CO_2 assimilation (i.e., ETR_A; **G)**, fraction of the total ETR used in photorespiration (i.e., ETR_P; **H)**, and ETR_A/ETR_P ratio **(I)** in young oil palm plants subjected to well-watered conditions (control) and one (WD 1x) and three (WD 3x) water deficit events. Data are the means ± SE (*n* = 5). Different capital letters denote significant differences between averages for the same treatment measured on different experimental days, and different small letters denote significant differences between different treatments compared on the same experimental day (Duncan's test, $P \leq 0.05$).

in WD 3x plants (Fig. 4H). Consequently, between days 7 and 21, WD 1x plants showed lower ETR_A/ETR_P than WD 3x plants, without differences between WD treatments on day 28 (Fig. 4I).

2.3.2. Rubisco final-activity, Rubisco activation state and triose-P utilization rate

The F values for Rubisco final-activity, Rubisco activation state, and TPU were significant for WR, T, and WR × T effects (Table 1). When the stressed plants were compared to their control counterparts between days 7 and 21, we observed that the final Rubisco activity was more significantly decreased in WD 1x than in WD 3x (Fig. 5A). Thus, the final Rubisco activities in WD 1x and WD 3x plants were 36% and 18% lower than in the control (Fig. 5A). On day 28, the final Rubisco activities in WD 1x and WD 3x plants were decreased by 39% and 17% relative to the control (Fig. 5A). Our data also showed that the Rubisco activation state was decreased by 15% in WD 1x plants on days 7 and 14, but it recovered to the control level on days 21 and 28 (Fig. 5B). Rubisco activation state did not differ between control and WD 3x plants in all evaluation times (Fig. 5B).

Seven days after withholding irrigation, the WD 1x and WD 3x plants showed slight decreases in TPU (around 7%) relative to control plants (Fig. 5C). However, when the plants were compared on days 14 and 21, we observed that TPU was more markedly decreased in WD 1x (31%) than in WD 3x (8%) plants (Fig. 5C). Such treatment differences were lacking under more prolonged drought (day 28) when TPU was similarly decreased by around 24% in both WD treatments (Fig. 5C).

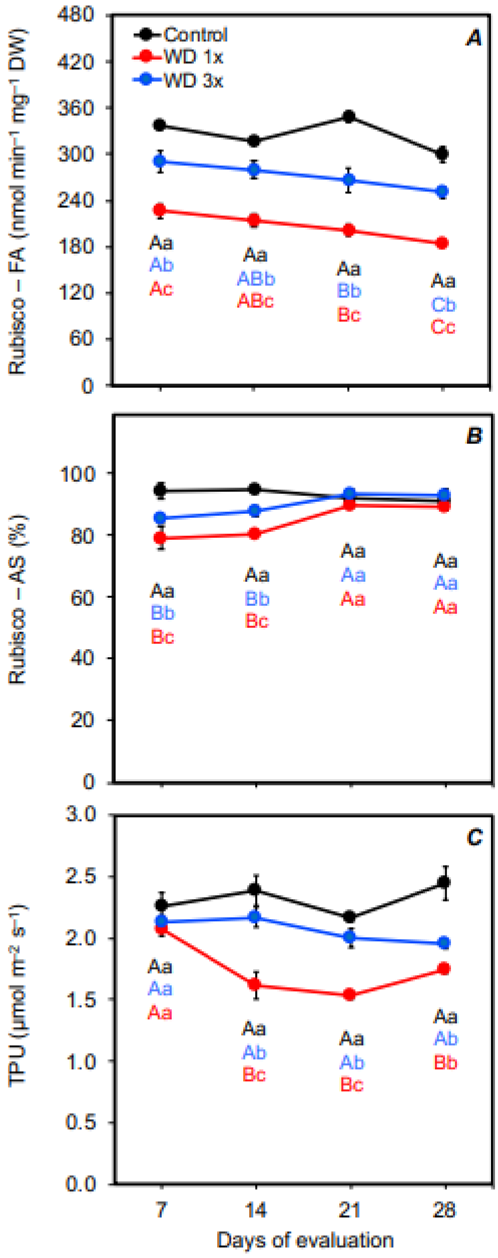

Fig. 5

Fig. 5. Time course of changes in Rubisco final-activity **(A)**, Rubisco activation state **(B)**, and triose-P utilization rate (TPU, **C**) in young oil palm plants subjected to well-watered conditions (control) and one (WD 1x) and three (WD 3x) water deficit events. Data are the means ± SE (*n* = 5). Different capital letters denote significant differences between averages for the same treatment measured on different experimental days, and different small letters denote significant differences between different treatments compared on the same experimental day (Duncan's test, $P \leq 0.05$).

2.3.3. Lipid peroxidation

The F values for MDA were significant for WR, T, and WR × T effects (Table 1). The WD by day 7 caused 82 % and 59 % increases in MDA concentrations in WD 1x and WD 3x plants relative to the control (Fig. 6). On subsequent days, we observed that lipid peroxidation was properly attenuated in WD 3x plants, but it was increased in WD 1x plants. Thus, in WD 1x plants between days 14 and 28, MDA concentrations were about two times greater than in control plants (Fig. 6).

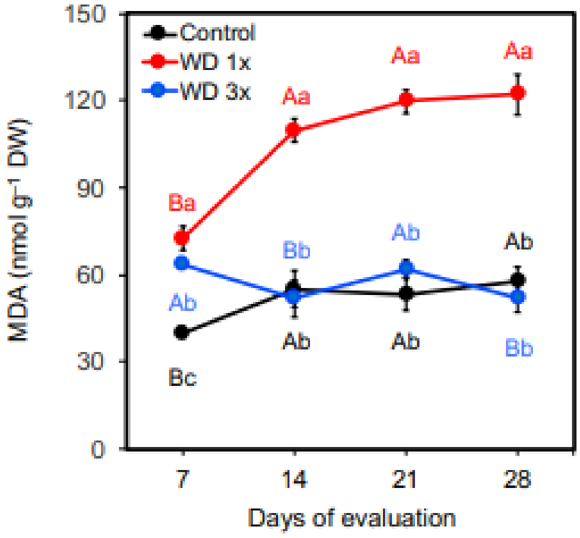

Fig. 6

Fig. 6. Time course of malondialdehyde (MDA) in oil palm plants subjected to well-watered conditions (control) and one (WD 1x) and three (WD 3x) water deficit events. Data are the means ± SE (n = 5). Different capital letters denote significant differences between averages for the same treatment measured on different experimental days, and different small letters denote significant differences between different treatments compared on the same experimental day (Duncan's test, $P \leq 0.05$).

2.4. Discussion

The physiological responses of oil palm plants subjected to a single water deficit event (WD 1x) showed the high sensitivity of this palm to soil water shortage. This inference is based on significant decreases in key leaf gas exchange variables (A, $g_{s,}$ and E) when the Ψ_{pd} in stressed plants reached about –1.81 MPa. These data corroborate with our prior findings, when we showed averages of A around zero in drought-stressed plants of two oil palm interspecific hybrids (Silva et al., 2016, 2017). Reductions in leaf gas exchange parameters were also observed in different oil palm plant materials subjected to different water deficit conditions (Bayona-Rodriguez et al., 2016; Rivera-Mendes et al., 2016; Bayona-Rodriguez and Romero, 2019; Najihah et al., 2019; Viana et al., 2019).

The regulation of A in WD-stressed plants is explained by several factors, including stomatal and non-stomatal mechanisms, such as changes in the photorespiration rate, activity of carbon metabolism enzymes, and changes in the expression of drought-responsive genes (Azzeme et al., 2016; Flexas et al., 2016; Wang et al., 2016; Urban et al., 2017). In oil palm, the WD-mediated decreases in A have been attributed to remarked decreases in gs, chlorophyll *a* and *b* degradation, photo-oxidative damages to PSII, lower Rubisco activity, and increasing photorespiration rate (Suresh et al., 2010; Azzeme et al., 2016; Silva et al., 2016). The inferences about rising photorespiration rates in WD-stressed oil palm have been indirectly proposed by determining GLO_x and CAT activities, while decreases in Rubisco activity have been related to possible decreases in [CO_2] in the stroma in response to lower g_s (Silva et al., 2016). Interestingly, our data showed that decreases in gs in WD 1x plants on day 7 were coincident with strong decreases in g_m and C_c, suggesting that stomatal limitations to photosynthesis are important traits in impairing photosynthesis in water-stressed plants of oil palm, as was also observed for other C_3 species under WD-conditions (Perdomo et al., 2017). Such stomatal limitations properly explain the lower V_{cmax}, initial (data not shown) and final Rubisco activities, and ETR_A in WD 1x plants as compared to control counterparts

Under WD conditions, the lower C_c and V_{cmax} may increase the O_2/CO_2 ratio in the stroma, leading to increases in the oxygenase activity of Rubisco, then triggering photorespiration rates in C_3 plants (Voss et al., 2013). Indeed, we showed that ETR_A markedly decreased in WD 1x plants relative to the control, and such decreases were parallel to increasing ETR_P and R_P in all evaluation times. Therefore, these data denote that photorespiration was markedly induced in WD 1x plants on day 7, and this remained greater than in control plants in all time points. Higher photorespiration rates were also observed in WD-stressed plants of *Jatropha curcas* (Silva et al., 2015), *Arachis hypogaea* (Pilon et al., 2018), and *Euterpe oleracea* (Oliveira et al., 2019), and these results confirm our first evidence for increasing photorespiration rates in WD-stressed oil palms (Silva et al., 2016). Therefore, we can assume that a greater photorespiration rate limits *A* in water-stressed palms to some extent (Takahashi and Badger, 2011; Voss et al., 2013).

Considering the pattern of decreases in *A*, V_{cmax}, and both initial (data not shown) and final Rubisco activities, we can infer that triose-P synthesis by the Calvin cycle was dramatically affected in WD 1x plants, particularly from day 7 onwards. The small amount of triose-P produced under stress conditions should be partitioned to support at least three pathways: (i) ribulose 1,5 bisphosphate (RuBP) regeneration to sustain Calvin cycle functioning, (ii) starch synthesis and its accumulation in the chloroplast during the day to support the night respiration, and (iii) sucrose synthesis, glycolysis, and other cytosolic pathways (McClain and Sharkey, 2019). Since J_{max} was lower in WD 1x than in control plants in all evaluation times, we can suggest that RuBP regeneration was negatively affected and this consequently contributed to limiting *A* in this species (Perdomo et al., 2017; Pilon et al., 2018). On the other hand, our prior work showed that starch synthesis in the chloroplast was substantially decreased in WD-stressed oil palm hybrids, while sucrose was undetected in leaf samples (Silva et al., 2017). Thus, we can presume that the synthesis of both carbohydrates was also limited in this experiment, as evidenced by the lower TPU in WD-stressed plants. Therefore, the lower TPU may have contributed to limiting *A* to some extent (Yang et al., 2016; McClain and Sharkey, 2019). Under WD conditions, it has been proposed that non-stomatal limitations of *A* have negative effects on the chloroplast electron transport chain (ETC_c). This is because the lower carboxylase activity of Rubisco induces lower ATP and NADPH consumption by the Calvin cycle. In this case, an accumulation of NADPH in the stroma implies reduced NADP availability to receive electrons from the photosystem I, leading to an over-reduction of the ETC_c (Medrano et al., 2002; Takahashi and Badger, 2011). To cope with this excess energy, plants can increase the rates of some protective mechanisms, such as (i) photorespiration (Takahashi and Badger, 2011; Voss et al., 2013; Silva et al., 2015), (ii) thermal energy dissipation, mainly as heat (Müller et al., 2001; Medrano et al., 2002; Oliveira et al., 2019), and (iii) cyclic electron transport (Takagi et al., 2016; Sunil et al., 2019). Nevertheless, part of the electrons from ETC_c can be directly transferred to molecular oxygen, leading to an overproduction of reactive oxygen species (ROS) in the stroma, triggering oxidative damages to proteins, lipids, and other cell components (Mittler, 2002). In this experiment, the over-reduction of ETC_c components in WD-stressed oil palms was evident by the lower averages of F_v/F_m, F'_v/F'_m, and q_L. Nevertheless, the stressed oil palms were able to increase photorespiration rate (as previously discussed) and increase the thermal energy dissipation, here evidenced as high NPQ averages. Comparatively, both mechanisms were also activated in WD-stressed plants of Euterpe oleracea (assai palm, Oliveira et al., 2019) and other oil palm plant materials (Suresh et al., 2010; Silva et al., 2016). Additional protection against oxidative stress may have been achieved by increased activities of antioxidant enzymes and changes in the ascorbate and glutathione pools, as previously shown in two oil palm hybrids subjected to progressive drought (Silva et al., 2016). Regardless of this plasticity, the decreased averages of F_v/F_m and F'_v/F'_m and increasing MDA levels in WD-stressed oil palms show that plants were affected by photo-oxidative damages to PSII and membrane lipids (Maia Júnior et al., 2019; Oliveira et al., 2019). Such damages occurred from day 7 (i.e., Ψ_{pd} = –1.81 MPa), reinforcing the idea that oil palm is very sensitive to soil water deficit.

Although several works concerning photosynthetic acclimation in response to repetitive WD events have been carried out for many plant species, this approach had not yet been examined in oil palm. Remarkably, our data show, for the first time, that oil palm plants subjected to repetitive WD events (WD 3x) can acclimate their photosynthetic machinery and attenuate oxidative damage to PSII and membrane lipids compared to plants exposed to a single WD event (WD 1x). This is supported by the higher averages of A, F_v/F_m, and F'_v/F'_m in WD 3x plants than in WD 1x, as well as by the smaller averages of MDA in the former. Similar acclimation of A was reported for other plant species in response to repetitive drought (Gomes et al., 2008; Menezes-Silva et al., 2017; Marcos et al., 2018). Conversely, non-significant differences in A between WD 1x and WD 2x treatments were observed, and for this reason, the following discussions will be centered on the comparison between the control, WD 1x, and WD 3x plants.

As previously discussed, the oil palms subjected to a single WD event showed marked decreases in *A* in response to stomatal and non-stomatal traits. The same pattern of limitations to *A* in response to a single WD event was observed when the plants were subjected to three WD events; however, they were at different magnitudes. Regarding the stomatal components, we observed that g_s varied similarly between WD 1x and WD 3x plants and, although a greater g_m was recorded in the WD 3x plants until day 21, the [CO_2] in the chloroplasts did not differ between WD treatments up to day 14. The absence of differences in the time course of g_s between WD 1x and WD 3x may be resulting from a similar pattern of abscisic acid synthesis between these treatments, as previously reported in *Saccharum* spp. (Marcos et al., 2018). Anyway, our results suggest that, regardless of the number of WD events experienced by the plants, the stomatal components played a similar role in *A* regulation until a Ψ_{pd} of around –1.81 MPa (i.e., up to day 14). After that (days 21 and 28), the increases in C_c regardless of low g_{s} and g_{m} in WD 1x and WD 3x plants suggest that limitations to *A* under more prolonged drought are more induced by non-stomatal traits than stomatal ones.

At least in part, the photosynthetic acclimation in response to repetitive WD events can be attributed to adjustments in the photochemical reactions because WD 3x plants showed higher averages of F_v/F_m, F'_v/F'_m (from day 7 onwards), and q_{L} (days 7 and 14) than there were in WD 1x plants. Comparatively, adjustments in F_v/F_m were observed in *Cocos nucifera* subjected to three WD cycles (Gomes et al., 2008), while in *C. canephora*, the exposure of plants to three WD events triggered adjustments in F_v/F_m, q_{P}, and ETR (Menezes-Silva et al., 2017). Acclimation of the photosynthetic machinery in oil palm was also shown by the differential adjustments in carboxylase and oxygenase activities of Rubisco in response to repetitive WD events. In this context, the highest averages of V_{cmax} (up to day 14) and initial and final Rubisco activities (up to day 21) in WD 3x plants indicated that Rubisco carboxylase activity in these plants was higher than in WD 1x counterparts. Greater Rubisco carboxylase activity in WD 3x plants can be attributed to a higher Rubisco activation state up to day 14 and the greater C_c in these plants from day 21 onwards. These differential responses explain the lowest ETR_P and R_P averages in WD 3x plants than there were in WD 1x plants and highlight that photorespiration adjustment is an important trait during the acclimation of oil palms to repetitive drought events, as was also observed *C. canephora* (Menezes-Silva et al., 2017).

The plasticity of oil palms in acclimating both *A* and R_P in response to repetitive WD events allows us to assume that triose-P production by the Calvin cycle was relatively higher in WD 3x plants than in WD 1x plants. Therefore, more substrate was likely available for RuBP regeneration, starch and sucrose synthesis, and cell respiration in the former. Since WD 3x plants showed the higher averages of J_{max} and TPU than WD 1x, a higher capacity to regenerate RuBP and use triose-P should be expected upon repetitive WD. Similar acclimation of J_{max} in response to repetitive drought was also observed in *C. nucifera* (Gomes et al., 2008). On the other hand, the highest TPU averages in WD 3x plants were coincident with lower R_d and R_P, while the opposite was observed in the WD 1x plants. Considering sucrose synthesis and glycolysis pathways compete for triose-P in the cytosol, these data suggest that down-regulation of respiration in response to repetitive WD events should favor greater sucrose synthesis in the leaves of WD 3x plants than in their WD 1x counterparts. Taken together, these differential adjustments in *A*, J_{max}, R_D, R_P, and TPU between WD 1x and WD 3x oil palm plants clearly show different adaptative mechanisms to cope with single and repetitive WD events.

Since WD 3x plants showed lower photorespiration and NPQ than WD 1x plants, then the lower incidence of oxidative damages to PSII and membrane lipids observed in the former may have resulted from a better adjustment in the antioxidant defense system. This possibility is supported by the positive modulation of antioxidant enzyme activities, as has been observed in other plant species subjected to repetitive WD events (Menezes-Silva et al., 2017; Alves et al., 2020; Lukić et al., 2020; Gadzinowska et al., 2021). For oil palm, positive modulations in the enzymatic and non-enzymatic antioxidant system have been reported in response to a single WD event (Silva et al., 2016); however, adjustments in response to repetitive WD events remains unknown and must be examined in future experiments.

Taking the data set obtained in this work, it was shown for the first time that oil palm plants submitted to repetitive WD events can memorize the effects of the first stress event. Consequently, oil palm plants subjected to repetitive WD events showed a better set of physiological responses to drought than those subjected to stress for the first time. This type of stress memorization has also been observed in water-stressed plants of *N. sylvestris* (Galle et al., 2009), *C. canephora* (Menezes-Silva et al., 2017), *Oryza sativa* (Li et al., 2019), and *Dipteryx alata* (Alves et al., 2020), among others. In general, stress memorization can be attributed to changes in the structure of the DNA, such as the occurrence of methylation and changes in the condensed state of chromatin (Avramova, 2015; He and Li, 2018). Therefore, when a plant is subjected to recurring stress events, the responsive genes are more easily accessed by transcription factors, which culminates in a greater and faster expression of these genes, changing the plant transcriptome, proteome, and metabolome (Avramova, 2015; Menezes-Silva et al., 2017; Li et al., 2019; Kim et al., 2020). These patterns of molecular alterations in response to repetitive stresses remain unknown in oil palm; however, our results suggest that changes in the expression of genes related to the control of stomatal opening, photochemical and biochemical reactions of photosynthesis, photorespiration, cytosolic and mitochondrial respiration, and antioxidant defense system must be crucial in this adaptative response. Therefore, further works should be performed by using molecular and physiological approaches to allow an integrated assessment of the memory of WD in oil palm.

2.5. Conclusions

We concluded that oil palms are very sensitive to single and repetitive WD events due to sharp plant dehydration, sharp decreases in A, and a higher incidence of oxidative damages to PSII and membrane lipid relative to control plants. The stomatal traits (lower g_s, g_m, and C_c) limited A more significantly, up to Ψ_{pd} of around –1.81 MPa (i.e., up to day 14), while non-stomatal limitations to A (lower Rubisco carboxylase activity and RuBP regeneration rate as well as higher photorespiration and respiration rates) were more significant with additional decreases in Ψ_{pd} (i.e., Ψ_{pd} lower than –1.81 MPa). Acclimation of the photosynthetic machinery in oil palm plants subjected to three WD events was achieved by increasing Rubisco carboxylase activity and J_{max} concomitantly to decreases in both photorespiration and respiration rates relative to plants subjected to a single WD event. Finally, acclimation of the photosynthetic machinery to repetitive WD events culminated in a lower level of oxidative damages to PSII and membrane lipids than in plants that were facing water deficit for the first time.

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3. CAPÍTULO 02. Eventos repetidos de deficit hídrico induzem ajustes nos mecanismos de dissipação de energia e na atividade de enzimas antioxidantes atenuando a ocorrência de danos oxidativos em palma de óleo**

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RESUMO

Este trabalho foi desenvolvido com o objetivo de avaliar a capacidade de ajustes no sistema antioxidante e subsequente atenuação da peroxidação lipídica em plantas de palma de óleo submetidas a eventos repetitivos de déficit hídrico. Para isso, um experimento foi conduzido com um tratamento controle (plantas sob irrigação plena) e dois tratamentos de estresse, em que as plantas foram submetidas a um (WD 1x) e três (WD 3x) eventos de déficit hídrico. As avaliações foram realizadas quando o potencial hídrico foliar na antemanhã (Ψ_{pd}) das plantas estressadas alcançou aproximadamente −2,5 MPa. As taxas de assimilação líquida do CO_2 (*A*) foram reduzidas em 99,3 % nas plantas WD 1x e 96 % nas plantas WD 3x quando comparadas ao controle, enquanto a condutância estomática foi reduzida em 94,6 % (WD 1x) e 97 % (WD 3x). A razão entre concentração de CO_2 subestomática e ambiente aumentou em média 35% nas plantas estressadas em relação às plantas controle, enquanto reduções expressivas na eficiência de carboxilação da Rubisco foram observadas nas plantas WD 1x e WD 3x. A máxima eficiência quântica do fotossistema II foi reduzida em 22,8% nas plantas WD 1x, enquanto as plantas WD 3x não diferiram do controle. O coeficiente de extinção não fotoquímico aumentou em 167,5% e 48,6% nas plantas WD 1x e WD 3x em relação ao controle e a razão entre a taxa de transporte aparente de elétron e a *A* foi aumentada em 426,5% e 200% nas plantas WD 1x e WD 3x. As atividades da dismutase do superóxido e redutase da glutationa aumentaram em 124% e 270% nas plantas WD 3x e 68,7% e 30% nas plantas WD 1x. As concentrações de peróxido de hidrogênio e aldeído malônico aumentaram em 127% e 110% nas plantas WD 1x, enquanto as plantas WD 3x e controle apresentaram médias similares para esses parâmetros. Conclui-se que a palma de óleo foi capaz ajustar seu sistema antioxidante para atenuar os danos oxidativos aos lipídeos de membrana quando submetidas a eventos repetitivos de déficit hídrico.

Palavras chaves: aclimatação fotossintética, danos foto-oxidativos, enzimas antioxidantes, memória ao déficit hídrico, peroxidação lipídica.

3.1. Introdução

O cultivo de palma de óleo (*Elaeis guineensis* Jacq.) no Brasil se concentram na região norte do país, particularmente no estado do Pará, o qual detém 98,4% das lavouras (IBGE, 2020) distribuídas em municípios onde o déficit hídrico anual é inferior a 100 mm (Villela et al., 2014). No entanto, a produção da palma de óleo é significativamente reduzida em regiões com déficit hídrico anual moderado (entre 100 e 350 mm) e severo (maior que 350 mm), inviabilizando os plantios de palma de óleo nessas áreas (Paterson & Lima, 2017). De fato, estudos com déficit hídrico prolongado têm demonstrado a redução no crescimento vegetativo, formação de cacho de frutas frescas e produtividade da palma de óleo (Noor & Harun, 2004; Putra et al., 2015; Silva et al., 2016; Culman et al., 2019), indicando que a espécie é muito sensível a esse estresse.

A duração dos períodos de déficit hídrico na Amazônia varia com as condições climáticas de cada local do bioma. Isto porque a grande extensão territorial da Amazônia (~6.700.000 km^2), faz com que as taxas de precipitação, a frequência e a distribuição das chuvas ao longo do ano sejam muito variáveis (Bastos, et al., 2001; Abrapalma, 2016). Além disso, as estiagens na Amazônia tendem a se agravar muito em anos de ocorrência de El Niño, o qual altera o padrão das chuvas na Amazônia (Cai et al., 2014; Raza et al., 2019). Então, mesmo nas áreas mais apropriadas ao cultivo da palma de óleo na Amazônia é possível que as plantas estejam propensas a períodos repetitivos de déficit hídrico.

Sob condições de déficit hídrico, as plantas ajustam sua fisiologia a fim de alcançar uma maior eficiência no uso da água e consequentemente maior tolerância das plantas ao déficit hídrico (Suresh et al., 2012., Costa et al., 2018., Viana et al., 2019).

Em palma de óleo, as plantas submetidas a um único evento de déficit hídrico apresentam significativas reduções no potencial hídrico foliar, nas taxas de assimilação líquida de CO_2 (A), condutância estomática ao vapor d’água (g_s), transpiração e eficiência instantânea do uso da água (Suresh et al., 2010; 2012; Jazzayeri et al., 2016) levando a alterações nas atividades de diferentes enzimas associadas ao metabolismo do carbono (e.g., ribulose1,5-bisfosfato carboxilase/oxigenase, Rubisco; desidrogenase do malato dependente de NAD^+ e oxidase do glicolato, GLOx; Silva et al., 2017) e sistema antioxidante (e.g., dismutase do superóxido, SOD; peroxidase do ascorbato, APX; catalase, CAT; e redutase da glutationa, GR; Silva et al., 2016).

Sob condições de déficit hídrico repetitivo, tem sido demonstrado que algumas espécies vegetais são capazes de ajustar seu metabolismo para suportar mais satisfatoriamente ao estresse quando comparadas às plantas que estão enfrentando o estresse pela primeira vez (Galle et al., 2011, Menezes-Silva et al., 2017, Nosalewicz et al., 2018, Virlouvet et al., 2018, Liu et al., 2019). Tal aclimatação é evidenciada por aumentos no conteúdo relativo de água e nas taxas de assimilação líquida de CO_2 (Ding et al., 2012; Galle et al., 2011; Menezes-Silva et al., 2017; Nosalewicz et al., 2018), decorrentes de uma regulação positiva da estrutura fotoquímica, como evidenciado pela integridade de clorofilas *a* e *b* (Fleta-Soriano e Munné-Bosch, 2016) e dos fotossistemas (Walter et al., 2011).

Tem sido também demonstrado que alterações no perfil metabólico influenciam na resposta diferencial das plantas expostas a períodos repetidos de déficit hídrico em relação às plantas expostas a um único evento de déficit hídrico. Essas alterações sugerem que ajustes metabólicos na respiração, fotorrespiração e fotossíntese desempenham importantes funções nos processos de aclimatação a múltiplos episódios de déficit hídrico. Em *Coffea canephora*, por exemplo, foram detectados aumentos nas atividades das enzimas Rubisco, fosfofructocinase, cinase do piruvato, aldolase e desidrogenase do malato, e reduções nas concentrações de carboidratos e aumentos nos níveis de aminoácidos e ácidos orgânicos, tais como glicina, fenilalanina, valina, isoleucina, isocitrato, succinato, fumarato e malato (Menezes-Silva et al., 2017). Então, tem sido proposto que plantas que experimentaram repetidos períodos de déficit hídrico e reidratação regulam o processo respiratório para direcionar os esqueletos de carbono para a produção de metabólitos essenciais para permitir a melhor performance durante o estresse se comparadas às plantas que sofrem um único evento de estresse (Galle et al., 2009; Hu et al., 2015; Menezes-Silva et al., 2017). Contudo, com a intensificação do déficit hídrico, a energia de excitação luminosa se torna superior à energia utilizada no processo fotossintético, inicialmente causando a redução na taxa de transporte de elétrons e posteriormente levando à saturação da cadeia transportadora de elétrons; provocando a produção de espécies reativas de oxigênio (ROS), que, caso não sejam devidamente detoxificados, induzirão reações oxidativas que levam à desestruturação do fotossistema II (PSII) e lipídeos de membrana (Foyer et al., 2012; Silva et al., 2016).

As plantas apresentam diversos mecanismos para remover ROS, os quais envolvem um sistema de enzimas e compostos não enzimáticos com ação antioxidante. Por exemplo, em folhas de *Carapa guianensis* (Carvalho et al. 2013), *C. canephora* (Lima et al. 2002) e *E. guineensis* (Silva et al. 2016) submetidas a um só evento de déficit hídrico, foi possível observar aumentos significativos nas atividades da SOD, CAT e APX paralelamente a reduções nas concentrações de aldeído malônico (MDA), composto indicativo de peroxidação lipídica (Cakmak e Horst 1991).

Recentemente, foi demonstrado que plantas de palma de óleo submetidas a três eventos de déficit hídrico apresentaram uma capacidade de aclimatação da maquinaria fotossintética por meio de ajustes na atividade da Rubisco, de enzimas relacionadas à fotorrespiração e ajustes na taxa de respiração quando comparadas às plantas submetidas a um evento de déficit hídrico (Wagner et al. 2021). Foi observado também que as plantas submetidas a três eventos de déficit hídrico apresentaram ajustes na máxima eficiência fotoquímica do PSII (F_v/F_m) e na concentração de aldeído malônico (MDA), cujos valores médios foram similares ás das plantas controle (plenamente irrigadas). Em contraste, as plantas de palma de óleo submetidas a um evento de déficit hídrico apresentaram reduções significativas na F_v/F_m e aumentos de MDA em relação às plantas controle (Wagner et al. 2021). Considerando-se que as plantas de palma de óleo submetidas a três eventos de déficit hídrico apresentaram uma menor incidência de danos oxidativos ao PSII e lipídeos de membrana que plantas submetidas ao déficit hídrico pela primeira vez, então pode-se estabelecer a hipótese de o déficit hídrico repetitivo é capaz de induzir ajustes nas atividades das enzimas antioxidantes, atenuando os efeitos oxidativos das ROS no FSII e peroxidação lipídica. Assim, este trabalho foi conduzido com o objetivo de investigar a capacidade de aclimatação do sistema antioxidante enzimático em plantas de palma de óleo submetidas a eventos repetitivos de déficit hídrico.

3.2. Material e métodos

3.2.1 Material vegetal e condições de crescimento

Mudas de palma de óleo de quatro meses de idade (*Elaeis guineensis* Jacq., Família Arecaceae, cultivar Tenera) foram dispostas em viveiro do Instituto de Ciências Agrárias (ICA) da Universidade Federal Rural da Amazônia (UFRA), Belém, Pará, Brasil (01º 27' 19” S e 48º 26' 19” O) e aclimatadas por 60 dias às condições climáticas locais. Em seguida, as mudas foram transferidas dos sacos para vasos de 50 L (49 cm × 59 cm, altura × diâmetro) preenchidos com uma mistura de Latossolo Amarelo distrófico e cama de aviário (3:1, v/v). A acidez do substrato foi corrigida pela aplicação de 30 g de calcário dolomítico (Plantei Garden Center Ltda ME, Salto Grande, Brasil) por vaso 30 dias antes do transplantio. Fertilizações com 10 g de NPK 15-11-21 (YaraMila PALMAE, Yara Brazil SA, Porto Alegre, Brasil), 2,5 g de sulfato de magnésio (YaraTera KRISTA MgS, Yara Brasil SA, Porto Alegre, Brasil) e 1 g de boro + cálcio (YaraVita CaBtrac, Yara Brasil SA, Porto Alegre, Brasil) por vaso a cada 30 dias contados a partir do transplantio. Além disso, fertilizações foliares com 20 g L^{-1} NPK 20-20-20 + micronutrientes (Plant-Prod 20-20-20 Classic, Master Plant-Prod Inc., Brampton, Canadá) foram realizadas em intervalos de 15 dias. A irrigação das plantas foi realizada diariamente, às 8:00 h (primeira irrigação) e às 17:00 h (segunda irrigação), visando manter o solo o mais próximo possível da capacidade de campo. O volume de água aplicado em cada tratamento foi estimado com base na variação da umidade do substrato entre irrigações, determinada segundo Klar et al. (1966).

3.2.2 Tratamentos e desenho experimental

As plantas foram cultivadas por 17 meses antes do início do experimento. A partir do stand de mudas disponível, foram selecionadas 15 plantas uniformes em altura (2,5 ± 0,2 m), circunferência do caule (65 ± 2 cm) e no número de folhas abertas (25 ± 1 unidades) e folhas flecha (3 ± 1 unidades). As mudas selecionadas foram distribuídas em três tratamentos: Controle, cujas plantas foram irrigadas diariamente durante todo o experimento; WD 1x, em que as plantas foram submetidas a um evento de déficit hídrico; e WD 3x, em que as plantas foram submetidas a três eventos de déficit hídrico (i.e., déficit hídrico repetitivo). Cada tratamento foi constituído de cinco repetições, sendo uma planta por vaso considerada repetição.

A indução do déficit hídrico foi realizada segundo Lopes Filho et al. (2021). Na primeira etapa, apenas as plantas do tratamento WD 3x estressadas, tendo sido as mesmas submetidas a dois eventos de déficit hídrico induzidos pela suspensão completa da irrigação até que o potencial hídrico foliar na antemanhã (Ψ_{pd}) atingisse −2,5 MPa. Após cada evento de estresse, as plantas foram submetidas à reidratação para a completa recuperação de seu status hídrico (Ψ_{pd} próximo ao das plantas controle). Ao final desta etapa, as plantas do tratamento WD 3x haviam sido submetidas a dois eventos de estresse, enquanto as plantas dos demais tratamentos permaneceram não estressadas. Na segunda etapa, a irrigação foi suspensa para as plantas dos tratamentos WD 1x e WD 3x, a fim de induzir o primeiro e o terceiro evento de déficit hídrico respectivamente; enquanto as plantas do tratamento controle permaneceram irrigadas conforme descrito anteriormente. As avaliações dos tratamentos foram realizadas quando o Ψ_{pd} das plantas estressadas atingisse −2,5 MPa. Na ocasião, foram avaliadas as trocas gasosas foliares, fluorescência da clorofila e amostragens para análises bioquímicas.

As avaliações e amostragens foram realizadas durante a manhã, entre 8:30 e 10:30 h. Nesse período, a temperatura do ar (T_{ar}) e a umidade relativa do ar (UR) foram determinadas por meio de um termohigrômetro e a radiação fotossinteticamente ativa (PAR) foi registrada por meio do sensor quântico conectado à câmara do analisador de gases a infravermelho. O déficit de pressão de vapor (DPV) foi estimado de acordo com Alvarenga et al. (2014). Esses dados foram obtidos em intervalos de 30 min. Assim, as médias de T_{ar} e UR foram respectivamente de 32,2 ± 0,20 ºC e 67,35 ± 0,45%, enquanto as médias de PAR e DPV foram respectivamente de 671,60 ± 32,52 μmol fótons m^{-2} s^{-1} e 1,63 ± 0,02 kPa.

3.2.3 *Potencial hídrico foliar*

As determinações de Ψ_{pd} foram realizadas entre 4:00 e 5:00 h, utilizando-se como amostra um folíolo da porção mediana da terceira folha a partir do ápice da planta. Após a coleta, o folíolo foi imediatamente encerrado em uma câmara de pressão do tipo Scholander (m670, Pms Instrument Co., Albany, EUA) para a determinação Ψ_{pd}.

3.2.4 Trocas gasosas e fluorescência da clorofila

As trocas gasosas e fluorescência da clorofila foram avaliadas simultaneamente, entre 8:30 e 9:30 h, utilizando-se um analisador de gases à infravermelho (LI-6400 XT; LI-COR Biosci. Inc., Nebraska, EUA) equipado com aparato de fluorescência (LI-6400-40, LI-COR Biosci. Inc., Nebraska, EUA). Como amostras, foram selecionados os folíolos opostos àqueles utilizados para a determinação do Ψ_{pd}. Os folíolos foram adaptados ao escuro por 30 min antes do início das leituras.

As trocas gasosas foram realizadas sob PAR de 1.100 µmol fótons m^{-2} s^{-1} e concentração de CO_2 de 400 µmol mol^{-1} (Silva et al. 2016, 2017). Foram determinadas a A, a g_s, a razão entre concentração de CO_2 subestomática e ambiente (C_i/C_a) e a eficiência instantânea da carboxilação (E_iC), esta última calculada pela razão entre A e C_i (Da Silva et al., 2015).

As determinações de fluorescência da clorofila foram realizadas a partir dos folíolos adaptados ao escuro. Primeiramente, foi aplicado um pulso de luz modular (0,03 µmol m^{-2} s^{-1} por 1 s) para determinar a fluorescência inicial (F_0), seguindo-se de um pulso de luz saturante (6000 µmol m m^{-2} s^{-1} por 0,8 s) para determinar a máxima fluorescência (F_m). Em seguida, uma luz actínica contínua (1200 µmol m^{-2} s^{-1}) foi aplicada por 480 s para determinar a fluorescência em estado estacionário (F_s). Na sequência, foram aplicados um pulso de luz branca saturante (6000 µmol m^{-2} s^{-1} por 0,8

s) e um pulso de luz vermelha distante (2 µmol m^{-2} s^{-1}) para obtenção da fluorescência máxima (F'_m) e do F_0 adaptado à luz (ou seja, F'_0). A F_v/F_m, a máxima eficiência fotoquímica do PS II no claro (F'_v/F'_m) e o coeficiente de extinção não fotoquímica (NPQ) foram estimados conforme Krause e Weis (1991). O coeficiente de extinção fotoquímica (q_P) foi calculado como $q_P = (F'_m - F_s)/(F'_m - F'_0)$ (Kramer et al., 2004). A taxa de transporte de elétrons (ETR) foi calculada como ETR = Φ_{PSII} × PPFD × f × α, em que PPFD é a densidade do fluxo de fótons fotossintéticos; f é o fator de compartimentação de energia entre PSII e PSI (f = 0,5) e α é a absorbância da folha pelos tecidos fotossintetizantes (α = 0,84; Maxwell e Johnson, 2000). O ETR/*A* foi calculado como um indicador de fotorrespiração (Bota et al., 2004).

3.2.5. Análises bioquímicas

Amostras contendo 30 discos foliares de 6,3 mm de diâmetro foram coletadas a partir do mesmo folíolo utilizado nas determinações de trocas gasosas e fluorescência e do folíolo adjacente a este. Os discos foram acondicionados em pacotes de papel alumínio e congelados em N_{Liq}. As amostras foram mantidas a −80 ºC até as análises.

Pigmentos cloroplastídicos

As amostras foram homogeneizadas em 5 mL de acetona 80% em presença de 0,01 g de $CaCO_3$. O homogenato resultante foi centrifugado a 12.000 × *g*, por 10 min a 10 ºC. O sobrenadante foi transferido para um balão volumétrico de 25 mL. Ao resíduo foi adicionado 5 mL de acetona 80%, seguindo-se de agitação em vórtex e centrifugação. O novo sobrenadante foi juntado ao primeiro e uma nova extração a partir do resíduo foi realizada por mais duas vezes seguindo a mesma metodologia. O volume final dos extratos reunidos no balão volumétrico foi ajustado para 25 mL com acetona 80% e a absorbância dos extratos foi determinada a 470 nm, 646,8 nm e 663,2 nm usando um espectrofotômetro UV-Visível. As concentrações de clorofila (Chl) *a* e *b* e carotenoides totais (CarTot) foram calculadas conforme descrito em Lichthenthaler (1987).

Enzimas antioxidantes

A dismutase do superóxido (SOD, EC 1.15.1.1) foi extraída em 2 mL de tampão de extração contendo tampão fosfato de potássio (TFK) 100 mM (pH 7,8), EDTA 0,1 mM, ditiotreitol (DTT) 1 mM, 2-mercaptoetanol 10 mM e Triton X-100 0,1% (v/v) (Giannopolitis e Ries, 1977). Após centrifugação a 15.000 × *g* por 15 min a 4 ºC, o sobrenadante foi coletado e a atividade da SOD foi determinada em 2 mL de meio de reação contendo TFK 52,5 mM (pH 7,8), NBT 0,075 mM, metionina 13 mM, riboflavina 2 μM (Giannopolitis e Ries, 1977) e 5 μL de extrato enzimático. O ensaio de atividade foi iniciado na presença de luz branca (122 μmol fótons m^{-2} s^{-1}) e finalizado após 10 min, desligando-se o fornecimento de luz. As absorbâncias das amostras foram determinadas a 560 nm. Uma unidade de SOD foi definida como a quantidade de enzima capaz inibir a fotorredução do NBT em 50% (Giannopolitis e Ries 1977).

A peroxidase do ascorbato (APX, EC 1.11.1.1) foi extraída em 2 mL de TFK 50 mM (pH 7,0) contendo ascorbato 20 mM, EDTA 2 mM e Triton X-100 0,1% (v/v). Após a centrifugação a 15.000 × *g* por 15 min a 4 ºC o sobrenadante foi usado para a determinação da atividade enzimática em 2 mL de meio de reação contendo TFK 50 mM (pH 7,0), ascorbato 0,5 mM, H_2O_2 0,1 mM e 15 μL de extrato. No ensaio, a atividade da APX foi determinada pela oxidação do ascorbato a 290 nm (Nakano e Asada, 1981).

A catalase (CAT, EC 1.11.1.6) foi extraída em 2 mL de TFK 50 mM (pH 7,0) contendo EDTA 2 mM e Triton X-100 0,1 % (v/v). Após centrifugação a 15.000 × *g* por 15 min a 4 ºC, o sobrenadante foi utilizado para o ensaio de atividade em 2 mL de meio de reação contendo TFK 50 mM (pH 7,0), H_2O_2 0,1 mM e 15 µL de extrato. A atividade da CAT foi determinada pela extinção do H_2O_2 a 240 nm (Havir e McHale 1987).

A redutase da glutationa (GR, EC 1.6.4.2) foi determinada conforme descrito em Pinheiro et al. (2004). A extração foi realizada em 2 mL de meio de extração contendo TFK 50 mM (pH 7,5), EDTA 2 mM, DTT 1 mM, 2-mercapetanol 10 mM, isoascorbato 10 mM e Triton X-100 0,1% (v/v). Após a centrifugação a 15.000 × *g* por 15 min a 4 ºC, uma alíquota de 15 µL do sobrenadante foi usado para a determinação da atividade enzimática em meio de reação constituído de TFK 50 mM (pH 7,5), glutationa oxidada 10 mM, $MgCl_2$ 3 mM e NADPH 0,15 mM, acompanhando-se a extinção de 6,22 mM^{-1} cm^{-1} a 340 nm (Pinheiro et al., 2004).

As atividades enzimáticas foram expressas em termos de concentração de proteínas solúveis totais dos extratos (Bradford 1976).

Peróxido de hidrogênio foliar

A concentração de peróxido de hidrogênio (H_2O_2) foi determinada de acordo com Velikova et al. (2000). Amostras foliares (500 mg PF) foram homogeneizadas em 5 mL de ácido tricloroacético (TCA) 0,1% (p/v) e o homogenato foi centrifugado a 12.000 × g por 15 min. Em seguida, uma alíquota de 0,5 mL do sobrenadante foi combinada a 0,5 mL de TFK 10 mM (pH 7,0) e 1 mL de iodeto de potássio 1 M. A absorbância da amostra foi obtida a 390 nm e a concentração de H_2O_2 obtida por meio da curva padrão.

Peroxidação lipídica

Foi determinada pelo método de Cakmak e Horst (1991), medindo-se a formação de intermediários reativos ao ácido 2-tiobarbitúrico (TBA) expressos em equivalentes de aldeído malônico (MDA), cuja concentração foi calculada com base no coeficiente de extinção molar do mesmo (155 mM^{-1} cm^{-1}). As absorbâncias específica e não específica das amostras foram determinadas a 532 e 600 nm, respectivamente.

3.2.6 Análise estatística

A normalidade dos dados foi testada pelo teste de Shapiro-Wilk e a homogeneidade das variâncias foi testada pelo teste de Levene. Os dados foram submetidos à ANOVA (teste F, $P \leq 0{,}05$) e as diferenças entre médias foram examinadas pelo teste de Duncan ($P \leq 0{,}05$). Os procedimentos estatísticos foram realizados usando software R (v. 3.4.1; R Core Team, 2016).

3.3. Resultados

3.3.1. Status hídrico, trocas gasosas e fluorescência da clorofila

Os dados referentes ao *status* hídrico, trocas gasosas e fluorescência da clorofila estão apresentados na Tabela 1. O Ψ_{pd} das plantas estressadas (WD 1x e WD 3x) foi reduzido para valores médios de -2.5 MPa. Em relação ao controle, foram observadas reduções significativas na A das plantas estressadas, sem diferenças entre plantas WD 1x e WD 3x. A g_s foi expressivamente reduzida nas plantas estressadas comparadas ao controle, mas tal redução foi ligeiramente mais significativa nas plantas do tratamento WD 3x que nas plantas do tratamento WD 1x. A C_i/C_a aumentou em média 35% nas plantas estressadas em relação às plantas controle, enquanto reduções significativas na E_iC da ordem de 99,7% e 96,1% foram observadas respectivamente nas plantas WD 1x e WD 3x em relação às plantas controle.

Sob condições de estresse, as plantas avaliadas diferiram na maioria dos parâmetros de fluorescência da clorofila *a* em relação ao controle (Tabela 1). Foi observado que o déficit hídrico causou diminuições significativas na F_v/F_m *apenas* nas plantas WD 1x (22,8 %). Por outro lado, as plantas estressadas apresentaram aumentos significativos no NPQ quando comparadas ao controle, contudo, esses aumentos foram mais expressivos nas plantas WD 1x (167,5%) que nas plantas WD 3x (48,6%).

As plantas submetidas a um ou três eventos de déficit hídrico apresentaram reduções similares no q_P (69%). Em relação ao controle, a ETR foi reduzida em 97% nas plantas WD 1x e 87% nas plantas WD 3x (Tabela 1). Consequentemente, os aumentos na ETR/*A* foram mais expressivos nas plantas WD 1x (427%) que nas plantas WD 3x (200%) quando comparadas ao controle.

Tabela 1. Status hídrico, trocas gasosas e fluorescência da clorofila em plantas de palma de óleo submetidas à plena irrigação (Controle) e a um (WD 1x) e três (WD 3x) eventos de déficit hídrico.

Parâmetros	Tratamentos		
	Controle	WD 1x	WD 3x
Ψ_{pd} (MPa)	-0,09 ± 0,01 a*	-2,51 ± 0,04 b	-2,49 ± 0,03 b
A (μmol m^{-2} s^{-1})	12,70 ± 0,39 a	0,08 ± 0,22 b	0,50 ± 0,31 b
g_s (mmol m^{-2} s^{-1})	202,11 ± 6,30 a	10,91 ± 2,67 b	5,95 ± 1,16 c
C_i/C_a (mol mol^{-1})	0,71 ± 0,04 a	0,91 ± 0,02 b	0,95 ± 0,01 b
E_iC (mol mol^{-1} m^{-2} s^{-1})	47,29 ± 1,50 a	0,13 ± 0,06 c	1,85 ± 0,03 b
F_v/F_m	0,83 ± 0,01 a	0,64 ± 0,04 b	0,82 ± 0,02 a
q_P	0,22 ± 0,03 a	0,07 ± 0,02 b	0,07 ± 0,01 b
NPQ	0,74 ± 0,07 c	1,98 ± 0,08 a	1,10 ± 0,02 b
ETR	65,2 ± 1,1 a	2,28 ± 0,39 c	8,46 ± 0,46 b
ETR/*A*	5,68 ± 0,30 c	29,91 ± 2,21 a	17,04 ± 1,40 b

*Os dados são as médias de cinco repetições ± erro padrão (EP). Letras minúsculas diferentes nas linhas denotam diferenças significativas entre médias de tratamentos (teste de Duncan, $P < 0{,}05$).

3.3.2. Pigmentos cloroplastídicos

Foi observado que o déficit hídrico causou uma redução considerável no teor de Chl *a* nas plantas WD 1x (55,9%), enquanto reduções menos expressivas foram observadas nas plantas WD 3x (29,7%) em relação a controle (Tabela 2). Em consequência, foram observadas diminuições significativas na razão Chl *a/b* nas plantas WD 1x (62,6%) e WD 3x (32,9%) em relação a controle (Tabela 2).

As concentrações de CarTot foram aumentadas nas plantas estressadas comparadas ao controle (Tabela 2). Esses aumentos foram de 226% nas plantas WD 1x e 55% nas plantas WD 3x (Tabela 2). Reduções de 89% e 46% na razão Chl *a+b*/CarTot foram observadas nas plantas WD 1x e WD 3x em relação a controle (Tabela 2).

Tabela 2. Valores de pigmentos cloroplastídicos em plantas de palma de óleo submetidas à plena irrigação (Controle) e a um (WD 1x) e três (WD 3x) eventos de déficit hídrico.

Parâmetros	Tratamentos		
	Controle	WD 1x	WD 3x
Chl *a* (g kg^{-1} PS)	15,19 ± 0,80 a*	6,69 ± 0,64 c	10,68 ± 0,69 b
Chl *a/b*	2,92 ± 0,12 a	1,09 ± 0,18 c	1,96 ± 0,08 b
CarTot (g kg^{-1} PS)	1,98 ± 0,18 c	6,47 ± 0,31 a	3,07 ± 0,02 b
Chl a+b / CarTot	14,14 ± 0,73 a	1,48 ± 0,03 c	7,62 ± 0,25 b

*Os dados são as médias de cinco repetições ± EP. Letras minúsculas diferentes nas linhas denotam diferenças significativas entre médias de tratamentos (teste de Duncan, $P < 0,05$).

3.3.3. H_2O_2 e peroxidação lipídica

As médias H_2O_2 e MDA (indicador de peroxidação lipídica) foram similares entre plantas controle e WD 3x (Tabela 3). Em contraste, aumentos de 127,1 % no H_2O_2 e 110,1 % no MDA foram observados nas plantas WD 1x em relação ao controle (Tabela 3).

Tabela 3. Concentrações de peróxido de hidrogênio (H_2O_2) e aldeído malônico (MDA) em plantas de palma de óleo submetidas à plena irrigação (Controle) e a um (WD 1x) e três (WD 3x) eventos de déficit hídrico.

Parâmetros	Tratamentos		
	Controle	WD 1x	WD 3x
H_2O_2 (μmol g^{-1} PS)	5,39 ± 0,35 b*	12,24 ± 0,44 a	4,87 ± 0,67 b
MDA (nmol g^{-1} PS)	58,10 ± 4,49 b	122,05 ± 6,75 a	52,28 ± 5,43 b

*Os dados são as médias de cinco repetições ± EP. Letras minúsculas diferentes nas linhas denotam diferenças significativas entre médias de tratamentos (teste de Duncan, *P* < 0,05).

3.3.4. Enzimas antioxidantes

Em relação ao controle, as atividades da SOD e GR foram respectivamente aumentadas em 124% e 270% nas plantas WD 3x e 69% e 30% nas plantas WD 1x (Tabela 4). Independente do número de eventos de estresse, aumentos significativos de mesma magnitude (253%) foram observados na atividade da CAT das plantas estressadas. Em contraste, a atividade de APX nas plantas WD 1x foi 61,2% maior que no controle, enquanto nas plantas WD 3x esse aumento foi de apenas 14 % (Tabela 4).

Tabela 4. Atividades da dismutase do superóxido (SOD), peroxidase do ascorbato (APX), catalase (CAT) e redutase da glutationa (GR) em plantas de palma de óleo submetidas à plena irrigação (Controle) e a um (WD 1x) e três (WD 3x) eventos de déficit hídrico.

Parâmetros	Tratamentos		
	Controle	WD 1x	WD 3x
SOD (mmol min^{-1} mg^{-1} prot.)	19,4 ± 0,6 c*	32,7 ± 1,3 b	43,5 ± 1,3 a
APX (mmol min^{-1} mg^{-1} prot.)	6,5 ± 0,2 c	10,6 ± 0,3 a	7,5 ± 0,1 b
CAT (mmol min^{-1} mg^{-1} prot.)	1,03 ± 0,13 a	3,62 ± 0,04 b	3,66 ± 0,34 b
GR (mmol min^{-1} mg^{-1} prot.)	0,10 ± 0,011 b	0,13 ± 0,008 b	0,37 ± 0,009 a

*Os dados são as médias de cinco repetições ± EP. Letras minúsculas diferentes nas linhas denotam diferenças significativas entre médias de tratamentos (teste de Duncan, *P* < 0,05).

3.4. Discussão

Independente do número de eventos de déficit hídrico aplicado, foi observado que os valores médios de Ψ_{pd} nas plantas estressadas foi igualmente reduzido para valores médios em torno de -2,5 MPa. Esse resultado indica que a perda de água pelas plantas foi similar ao final do período experimental de 28 dias. A desidratação das plantas estressadas WD 1x e WD 3x ocorreu mesmo com as reduções expressivas g_s, que supostamente limitou a transpiração das plantas estressadas. Os resultados obtidos sugerem que os eventos de déficit hídrico repetitivo não dispararam ajustes fisiológicos relacionados à manutenção da turgescência foliar, como também observado em *Nicotiana sylvestris* (Galle et al., 2009), *Coffea canefora* (Meneses-Silva et al., 2017) , *Zea mays* (Virlouvet et al. 2018), *Alopecurus pratensis* (Lukić et al., 2020), estes estudos demonstraram que as plantas podem apresentar uma aclimatação ao déficit hídrico, através de um sistema antioxidante mais robusto, melhorando seu desempenho quando submetidas a eventos repetitivos de déficit hídrico.

No presente estudo, as expressivas reduções na *A* das plantas WD 1x e WD 3x indicam que a palma de óleo possui um aparato fotossintético consideravelmente sensível às reduções de Ψ_{pd}. Além disso, as reduções na *A* nas plantas WD 1x e WD 3x em magnitudes similares em relação ao controle sugerem que o déficit hídrico repetitivo não induziu uma resposta favorável à fotossíntese ao final do período experimental. Ressalta-se, porém, que o déficit hídrico aplicado neste trabalho pode ser considerado muito severo para esta espécie, visto que as reduções na *A* das plantas WD 1x e WD 3x foram superiores a 90% em relação ao controle, com valores médios próximos a zero. As reduções na *A* observadas nas plantas WD 1x e WD 3x neste trabalho corroboram com nossos resultados anteriores, quando observamos reduções de 90 a 95% na *A* de dois híbridos de *E. guineensis* submetidos a um evento de déficit hídrico caracterizado pela redução do Ψ_{pd} para –2,0 MPa (Silva et al., 2016; 2017). Reduções expressivas em *A* (99%) foram também observadas em dois híbridos interespecíficos de *E. oleifera* × *E. guineenses* (OG) materiais vegetais de palma de óleo submetidos a um evento de déficit hídrico caracterizado pela redução do conteúdo relativo de água para valores em torno de 20% em 15 dias de estresse (Tezara et al., 2021). A sensibilidade do aparato fotossintético da palma de óleo a um evento de déficit hídrico foi também reportada para outros materiais vegetais desta palmeira submetidos a diferentes disponibilidades de água no solo (Bayona-Rodríguez e Romero, 2019; Najihah et al., 2019; Oliveira et al., 2020). Portanto, independente do número de eventos de déficit hídrico que a palma de óleo experimenta, as expressivas reduções na *A* são indicativos da alta sensibilidade da espécie ao déficit hídrico.

Lopes Filho et al. (2021) demonstraram que as diferenças nas respostas fotossintéticas de plantas de palma de óleo submetidas a um, dois e três eventos de déficit hídrico foram mais evidentes até um Ψ_{pd} para –2,0 MPa (Wagner et al., 2021). Nesse caso, as plantas submetidas a três eventos de déficit hídrico apresentaram valores de *A* significativamente maiores que plantas submetidas a apenas um evento de déficit hídrico (Lopes Filho et al., 2021). A evidente capacidade de aclimatação fotossintética da palma de óleo em resposta aos eventos de déficit hídrico repetitivo é perdida sob déficit hídricos mais prolongados (Lopes Filho et al., 2021), o que suporta a ausência de diferenças na *A* entre plantas WD 1x e WD 3x neste trabalho.

As reduções na g_s observadas nas plantas WD 1x e WD 3x deste trabalho podem ter resultado em uma maior resistência ao influxo de CO_2 às folhas, o que, associado a problemas de ordens difusivas, devem ter resultado em menores concentrações de CO_2 nos cloroplastos, particularmente nos sítios de carboxilação da Rubisco, contribuindo para a diminuição da *A* (Flexas et al., 2004, 2007; Silva et al. 2016). De fato, em palma de óleo, foi recentemente demonstrado que o déficit hídrico por uma ou mais vezes diminuiu a condutância mesofílica ao CO_2 e a concentração cloroplastídica de CO_2, resultando em uma menor velocidade de carboxilação da Rubisco, menor estado de ativação da Rubisco e menor atividade final dessa enzima (Wagner et al., 2021). É nótorio que as reduções na g_s observadas nas plantas WD 1x e WD 3x do presente estudo foram concorrentes às reduções na E_iC, ao mesmo tempo em que a fotorrespiração pode ter sido induzido nessas plantas, conforme evidenciado pelos aumentos no ETR/*A*. Então, além dos fatores de ordem estomática, a menor eficiência de carboxilação da Rubisco e os aumentos na fotorrespiração são fatores que atuam na diminuição da *A* nesta palmeira, confirmando resultados previamente reportados por outros autores (Silva et al. 2016, 2017; Lopes Filho et al., 2021).

Um dos problemas associados às limitações estomáticas da fotossíntese e à menor disponibilidade de CO_2 para a Rubisco é o desequilíbrio entre as etapas fotoquímica e bioquímica da fotossíntese que reduzem a carboxilação (Lawlor e Cornic, 2002; Chaves et al., 2003) e causam danos oxidativos, que podem levar à morte celular (Baker et al., 2007; Lawlor e Tezara, 2009). Esse desequilíbrio ocorre porque a menor atividade carboxilativa da Rubisco resultará em um menor consumo de ATP e NADPH produzidos pela etapa fotoquímica, levando a um acúmulo transitório desses compostos no estroma. Uma vez que os fotossistemas continuam a receber energia luminosa, então o transporte de elétrons deve ser mantido em alguma extensão. De fato, as reduções significativas observadas no q_P das plantas WD 1x e WD 3x neste trabalho evidenciam que a dissipação de energia luminosa para a realização de processos fotoquímicos foi muito prejudicada, e isto consequentemente colaborou para a redução da ETR no PS II em ambos os tratamentos. Reduções no q_P e ETR foram previamente reportadas em diversos materiais vegetais de palma de óleo submetidos a um evento de déficit hídrico (Liberato et al., 2006; Suresh et al., 2010, 2012; Bayona-Rodríguez et al., 2016).

Com a menor disponibilidade de NADP no estroma, é possível que os elétrons do PS I sejam doados diretamente ao O_2, produzindo o ânion superóxido ($O_2^{\bullet-}$) (Asada, 1999). Além do $O_2^{\bullet-}$, o oxigênio singleto ($^1O_2$) é potencialmente produzido nos cloroplastos em decorrência de eventos de fotoinibição e do próprio transporte de elétrons no FS II, enquanto o H_2O_2 é produzido pela detoxificação do $O_2^{\bullet-}$ pela SOD no stroma e pela ação da GLOx nos peroxissomos (Asada, 1999, 2006; Rhoads et al. 2006). Em conjunto, essas ROS podem proporcionar a um estresse oxidativo capaz de oxidar proteínas, lipídeos de membranas, DNA, RNA, dentre outros, causando danos que podem levar à desestruturação de membranas e perda de compartimentalização celular (Polle, 2001; Foyer, 2002; Reddy et al., 2004; Demidchik, 2015). A ocorrência de estresse oxidativo mediado pelo déficit hídrico tem sido extensivamente demonstrada em palma de óleo (Suresh et al., 2012; Cha-um et al., 2013; Silva et al., 2016; Najihah, et al., 2019) e em várias outras espécies (Lima et al., 2002; Carvalho et al., 2013; Meneses-Silva et al., 2017; Oliveira, et al., 2019; Lukić et al., 2020).

No presente estudo, as maiores médias de NPQ nas plantas WD 1x que nas plantas WD 3x indicam uma maior capacidade de dissipação de energia luminosa na forma de calor nas primeiras (Müller et al., 2001). Embora esse mecanismo alivie a pressão na cadeia de transporte de elétrons cloroplastídica, tem sido observado que a dissipação térmica, sozinha, não é suficiente para prevenir danos foto-oxidativos (Liberato et al., 2006; Suresh et al., 2010, 2012; Meneses e Silva et al., 2017). Por outro lado, a maior concentração de H_2O_2 nas plantas WD 1x evidenciam um maior potencial oxidativo que nas plantas WD 3x. Essas diferenças de concentração no H_2O_2 devem ter sido decorrentes tanto do aumento da fotorrespiração quanto do aumento da atividade da SOD nessas plantas. Interessante ressaltar que a maior atividade da (SOD, GR e CAT) nas plantas WD 3x em relação às plantas WD 1x indicam que as primeiras apresentaram uma maior eficiência em detoxificar ROS. Portanto, os resultados obtidos mostram claramente que apesar das plantas de palma de óleo que estavam experimentando o estresse pela primeira vez (WD 1x) ativarem de forma mais contundente os seus mecanismos de defesa aos excessos de energia (dissipação térmica e fotorrespiração) e eliminação enzimática de ROS pelo aumento de APX. Ainda assim, as plantas WD 1x apresentaram maior degradação de Chl *a*, maior incidência de danos ao PS II (evidenciado pela redução na F_v/F_m) e maior ocorrência de peroxidação lipídica que as plantas WD 3x. Diante disto, pode-se inferir que as plantas WD 3x apresentaram uma capacidade de resposta aos efeitos do déficit hídrico mais apropriada que as plantas WD 1x, i.e., foram aptas a ajustar seu metabolismo para lidar com os efeitos adversos do estresse.

3.5. Conclusão

As plantas submetidas a três eventos de déficit hídrico apresentaram melhores ajustes nas atividades de enzimas antioxidantes (SOD, GR e CAT) que plantas submetidas a um só evento de estresse. Consequentemente, as plantas WD 3x conseguiram atenuar de uma forma mais satisfatória os danos oxidativos mediados pelo déficit hídrico que as plantas WD 1x.

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6. Conclusão geral

O conjunto de dados obtidos permite concluir que as plantas de palma de óleo sofreram fortes diminuições na *A* mediadas por fatores estomáticos e não estomáticos, evidenciando a forte sensibilidade da espécie ao déficit hídrico.

A palma de óleo é capaz de ajustar seu metabolismo fotossintético, fotorrespiratório e repois foram capazes de aclimatar seu maquinário fotossintético, assim como modular seu sistema antioxidante atenuando danos axidativos e melhorando seu desempenho quando submetidas a eventos repetitivos de déficit hídrico.