FÁBIO PINTO GOMES

TROCAS GASOSAS EM COQUEIRO ANÃO-VERDE SUBMETIDO A CICLOS DE DEFICIÊNCIA HÍDRICA

Tese apresentada à Universidade Federal de Viçosa como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal para a obtenção do título de *Doctor Scientiae*.

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APROVADA: 02 de fevereiro de 2006.

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À minha esposa Luana
Aos meus filhos Vítor e Júlia
Pelo sentido que dão às nossas vidas.

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BIOGRAFIA

Fábio Pinto Gomes nasceu a 10 de julho de 1970 em Salvador, BA. Concluiu os cursos de Graduação em Agronomia em janeiro de 1993 pela Universidade Federal de Viçosa e, de Mestrado em Biologia/Botânica, em julho de 1996 pela Universidade Federal da Bahia. A partir de fevereiro de 1998 iniciou os trabalhos como Professor de bioquímica na Universidade Estadual de Santa Cruz, em Ilhéus, BA. Em abril de 2002 iniciou o curso de Doutorado na Universidade Federal de Viçosa, Programa de Pós-Graduação em Fisiologia Vegetal do Departamento de Biologia Vegetal.

SÍMBOLOS E ABREVIAÇÕES

Símbolo/Abreviação	Variáveis/Parâmetros (unidade)
q	Termo de convexidade (adimensional)
[ABA]	Concentração de ácido abscísico (nmol ABA g^{-1} matéria seca)
A	Taxa de fotossíntese líquida (μmol CO_2 m^{-2} s^{-1})
A/gs	Eficiência intrínseca de uso da água (μmol CO_2 $mmol^{-1}$ H_2O)
A_{maxCO2}	Taxa fotossintética saturada por CO_2 (μmol CO_2 m^{-2} s^{-1})
A_{maxPAR}	Taxa fotossintética saturada pela irradiância (μmol CO_2 m^{-2} s^{-1})
Ca	Concentração atmosférica de CO_2 (μmol CO_2 mol^{-1} ar)
CE	Eficiência de carboxilação (μmol CO_2 $mmol^{-1}$ CO_2)
Ci	Concentração de CO_2 nos espaços intercelulares (μmol CO_2 mol^{-1}
DMSO	Dimetilsulfóxido
E	Taxa de transpiração (mmol H_2O m^{-2} s^{-1})
F_0	Fluorescência mínima (basal) (unidades relativas)
Fv/Fm	Eficiência quântica máxima do fotossistema II
gs	Condutância estomática ao vapor de água (mol H_2O m^{-2} s^{-1})
J	Taxa de transporte de elétrons (μmol elétrons m^{-2} s^{-1})
JGD	Coqueiro Anão-Verde de Jiqui, Rio Grande do Norte
J_{max}	J saturada pela irradiância (μmol elétrons m^{-2} s^{-1})
Lm	Limitação mesofílica da fotossíntese (%)
L_S	Limitação estomática relativa da fotossíntese (%)
OA	Ajustamento osmótico (= Ψ_S(controle) – Ψ_S(estressada), MPa)
$PPFD$	Fluxo de fótons fotossinteticamente ativos (μmol fótons m^{-2} s^{-1})
R_{d}	Taxa de respiração no escuro (μmol CO_2 m^{-2} s^{-1})
R_{L}	Taxa de respiração no luz (μmol CO_2 m^{-2} s^{-1})
UGD	Coqueiro Anão-Verde de Una, Bahia
VPD	Diferença de pressão de vapor de água entre a folha e o ar (kPa)
WS	Plantas estressadas
WW	Plantas controle
Γ_{CO2}	Ponto de compensação de CO_2 (μmol CO_2 mol^{-1} ar)
Γ_{PAR}	Irradiância de compensação (μmol fótons m^{-2} s^{-1})
Ψ_{PD}	Potencial hídrico foliar ‘pre-dawn’ (MPa)
Ψ_{S}	Potencial osmótico (MPa)
α	Eficiência quântica aparente da assimilação (μmol CO_2 $mmol^{-1}$

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RESUMO

GOMES, Fábio P., D.S. Universidade Federal de Viçosa, fevereiro de 2006. **Trocas gasosas em coqueiro Anão-Verde submetido a ciclos de deficiência hídrica.** Orientador: Marco Antônio Oliva Cano. Conselheiros: Helio Garcia Leite e Marcelo Schramm Mielke.

Com o objetivo de avaliar as trocas gasosas em nível foliar, as relações hídricas, a capacidade de ajuste osmótico e a regulação estomática em plantas jovens de coqueiro Anão-verde submetidas à deficiência hídrica, realizou-se um experimento em casa de vegetação utilizando dois ecotipos de coqueiro anão-verde do Brasil: Anão-Verde de Una (UGD), Bahia e Anão-Verde de Jiqui (JGD), Rio Grande do Norte. Após 12 meses de cultivo em tanques de fibra contendo 0,145 m^3 de uma mistura solo:areia (2:1), parte das plantas foi submetida a três ciclos de deficiência hídrica. Cada ciclo consistiu em suspender a irrigação até que a taxa fotossintética (A) atingisse valor próximo à zero, retomando as irrigações até que A recuperasse a, no mínimo, 85% em relação às plantas controle. Reduções de A, condutância estomática ao vapor de água (gs) e transpiração (E) a valores quase nulos foram observadas quando o potencial hídrico foliar medido antes do nascer do sol (Ψ_{PD}) decresceu a -1,2 MPa. Adicionalmente, gs e E decresceram antes que A. Os valores limite de gs e Ψ_{PD} abaixo dos quais fatores não-estomáticos passaram a limitar a assimilação de carbono foram, respectivamente, 0,035 mol m^{-2} s^{-1} e -1,0 MPa. Observou-se reduções em gs e incrementos na concentração de ácido abscísico ([ABA]) antes que fossem detectados decréscimos expressivos em Ψ_{PD}, indicando que gs é controlada por ABA sob condições de estresse moderado. Aumentos expressivos na eficiência intrínseca de uso da água (A/gs), sem decréscimos significativos em A, sob condições de estresse moderado, também foram observados. O coqueiro Anão-Verde mostrou ajustamento osmótico de 0.05 a 0.24 MPa, sem, no entanto, evitar os efeitos da deficiência hídrica severa no crescimento. Aumento significativo na concentração de prolina (2 a 3 vezes o valor medido nas plantas controle) foi observado nos dois ecotipos, o qual parece ter uma função relacionada mais à proteção das membranas e manutenção da integridade de macromoléculas do que ao ajuste osmótico. Nos três ciclos, após reidratação por quatro dias, Ψ_{PD} aumentou de -1,20 para -0,15 MPa, em média, sendo observadas apenas pequenas diferenças em Ci/Ca. Entretanto, A não recuperou totalmente para valores comparáveis aos das plantas controle. Nesse momento, as taxas de fotossíntese saturadas por CO_2 (A_{maxCO2}) e pela radiação fotossinteticamente ativa (*PPFD*) (A_{maxPAR}), a eficiência de carboxilação (CE), a eficiência quântica máxima do PSII (Fv/Fm) e a taxa máxima de transporte de elétrons (J_{max}) foram significativamente menores nas plantas estressadas. As limitações estomáticas (L_S) variaram de 16 a 20% nas plantas controle, sem diferenças significativas entre os tratamentos e entre os ecotipos. As limitações mesofílicas foram significativamente maiores no JGD no ciclo 1, mas decresceram a valores significativamente menores do que no UGD nos dois outros ciclos. Os resultados mostraram que, após quatro dias de reidratação, as limitações estomáticas das plantas estressadas foram comparáveis às das plantas controle (~20%), sendo a recuperação incompleta de *A* devida a fatores não-estomáticos. Os ecotipos de coqueiro anão-verde, estudados no presente trabalho, mostraram baixa tolerância à deficiência hídrica, visto que tiveram as trocas gasosas e o crescimento severamente afetados, apesar de mostrarem características de evite da desidratação por meio do controle estomático da transpiração. Entretanto, o JGD mostrou algumas vantagens quando comparado ao UGD, como maior eficiência de uso da água ao final do experimento e maior capacidade de acúmulo de prolina. Além disso, durante o período de recuperação, observou-se menor limitação mesofílica, maior eficiência quântica aparente e sinais de aclimatação da capacidade fotossintética (menor efeito da seca na concentração de pigmentos foliares, em $\mathrm{A}_{\mathrm{maxCO2}}$ e CE) no JGD após sucessivos ciclos de deficiência hídrica. Três modelos frequentemente utilizados para descrever empiricamente a resposta de *A* a *PPFD* (hipérboles retangular e não-retangular e exponencial) foram comparados utilizando dados experimentais obtidos em plantas adultas e jovens de UGD, cultivadas em cinco diferentes locais. Os resultados mostraram que os três modelos foram considerados quantitativamente adequados. Entretanto, a hipérbole retangular superestimou a taxa fotossintética saturada por *PPFD* e a eficiência quântica aparente. A hipérbole não-retangular foi menos adequada para estimar os parâmetros fotossintéticos universalmente, mas apresenta como vantagem qualitativa a presença do termo de convexidade que representa a razão entre as resistências física e total à difusão de CO_2 para os cloroplastos. Os resultados permitem sugerir o modelo exponencial como uma opção simples, rápida, quantitativa e qualitativamente adequada para acessar as informações das curvas de resposta de *A* a *PPFD*.

ABSTRACT

GOMES, Fábio P., D.S. Universidade Federal de Viçosa, February, 2006. **Leaf gas exchange in Green-Dwarf coconut submitted to cycles of water deficit.** Advisor: Marco Antônio Oliva Cano. Committee Members: Helio Garcia Leite and Marcelo Schramm Mielke

With the objective of evaluating the impact of water stress on the photosynthetic gas exchange, water relations, osmotic adjustment capacity and stomatal regulation in young plants of Brazilian Green Dwarf (GD) coconut, a pot experiment was conducted under greenhouse conditions. Plants of two ecotypes from Una, Bahia State (UGD) and from Jiqui, Rio Grande do Norte State (JGD) were submitted to three successive drought cycles by suspending the irrigation until the net photosynthetic rate (*A*) approached zero and rewatering until recovery of the assimilation rates to, at least 85% of the control. Leaflet *A*, stomatal conductance to water vapour (*gs*) and transpiration (*E*) were negligible when the pre-dawn leaf water potential (Ψ_{PD}) reached about -1.2 MPa, being observed reduction in *gs* and *E* before significant decreases in *A*. It was observed that *gs* = 0.04 mol m^{-2} s^{-1} and Ψ_{PD} = -1.0 MPa were the threshold values bellow which the intercellular CO_2 concentration (*Ci*) increased and the intrinsic water use efficiency (*A*/*gs*) decreased, indicating that non-stomatal factors are limiting *A*. Decreases in *gs* and increases in leaflet abscisic acid concentration ([ABA]) were observed before significant changes in the leaf water status, indicating that *gs* was controlled by ABA under mild water stress. *A*/*gs* was improved at mild water deficit without significant impairment of *A*. Green dwarf coconut palm showed osmotic adjustment of 0.05 to 0.24 MPa in response to water deficit, which did not avoid impairment of growth under severe water deficit. Significant accumulation of proline (two to three times higher than their controls) in leaflets was observed in the two ecotypes, which was more related to protective rather than to osmoregulatory functions. Upon rewatering for four days, pre-dawn leaf water potential of stressed plants increased from -1.20 to -0.15 MPa on average and only small differences in *Ci*/*Ca* were detected but the photosynthesis rate (*A*) was not completely recovered to the control values. Light (A_{maxPAR}) and CO_2-saturated (A_{maxCO2}) *A*, carboxylation efficiency (CE), maximum quantum yield of PSII (Fv/Fm) and maximum electron transport rate ($J_{\max}$) were significantly lower in stressed plants at the three cycles. Relative stomatal limitation ranged from 16 to 20% in irrigated plants without significant differences between treatments and between ecotypes. The relative mesophyll limitation was significantly higher in JGD in the cycle 1 but it decreased to values significantly lower than in UGD in the other two cycles. The results showed that, after four days of rewatering, stomatal limitations of stressed plants were comparable to that of control (~20%), being the incomplete recovery of A after drought stress due to non-stomatal factors. The two ecotypes of Brazilian Green-Dwarf coconut studied in the present experiment showed low tolerance to water deficit, as indicated by decreases in photosynthetic gas exchange, as well as marked reduction in growth parameters, in spite of showing a dehydration avoidance mechanism through early stomatal closure. However, slight differences among then, with respect to the response to treatments were observed, the JGD showing higher water use efficiency after repeated cycles of water stress, as well as, higher proline accumulation capacity. In addition, during the recovery phase, the ecotype JGD showed smaller relative mesophyll limitation, faster recovery of apparent quantum efficiency and signs of acclimation of the photosynthetic capacity after repeated drying/recovery cycles. Experimental data of irradiance-response curves of photosynthesis, obtained from independent experiments with the UGD ecotype, were used to fit three empirical models with the aim of comparing their adequacies. The non-rectangular and rectangular hyperbolas and the exponential models were compared. All three models were quantitatively adequate to describe the irradiance response of photosynthesis in dwarf coconut. The photosynthetic parameters showed good correlation with the measured values when estimated by the rectangular hyperbola and exponential but the former overestimated the light-saturated rate and the apparent quantum yield of photosynthesis. The NRH was less adequate for estimate the light-saturated photosynthesis and dark respiration universally, but could be used for research purposes since it contains an important qualitative parameter, the convexity term relating physical to total resistances to CO_2 diffusion into the chloroplasts. The results allow suggesting the exponential model as a rapid, simple, quantitatively and qualitatively adequate option for accessing information from the photosynthetic irradiance-response in dwarf coconut palm.

INTRODUÇÃO GERAL

A espécie *Cocos nucifera* L. (Palmae), cuja região de origem mais aceita é o sudeste asiático, pode ser encontrada na região litorânea e no interior de quase todos os países tropicais. Sua ampla distribuição tem sido favorecida pelas inúmeras possibilidades de uso da planta, bem como por sua adaptabilidade a diferentes condições ecológicas (Ohler, 1999). Apresenta duas variedades botânicas a gigante (*C. nucifera* L. ‘*typica’*) e a anã (*C. nucifera* L. ‘*nana’*). Dentro de cada variedade, estão os cultivares, ou seja, materiais característicos de uma região em particular, onde têm sido cultivados por muito tempo, como, por exemplo, os anões da Malásia e do Brasil, ou os gigantes da costa Leste ou da costa oeste, na África. Podem ser encontrados ainda, em cada cultivar, membros que diferem uns dos outros em uma ou em algumas características, como tamanho e cor dos frutos, constituindo as formas ou ecotipos (Satyabalan, 1997). A variedade anã, sobretudo o cultivar anão-verde, é a mais utilizada para a produção de água de coco, principalmente por sua maior precocidade, maior produção de frutos, menor porte e pelo melhor sabor da água de seus frutos. Estima-se que a área cultivada com coqueiro anão no Brasil seja de 60 mil hectares, só com os plantios irrigados, sendo o Estado do Espírito Santo o líder com 14 mil hectares, seguido pela Bahia com 12 mil hectares. O consumo de água de coco envasada cresce a uma taxa de 20% ao ano, uma demanda que está longe de ser atendida pela oferta atual. Além disso, há uma forte tendência de se buscar o mercado internacional, principalmente durante o verão do hemisfério norte quando a demanda interna é reduzida devido ao inverno (Cuenca et al., 2002).

O coqueiro é considerado altamente sensível ao déficit hídrico em termos de assimilação de CO_2, produção e partição de matéria seca (Rajagopal e Kasturi Bai, 1999), sendo a sua produção significativamente diminuída pela duração do período seco, sobretudo nos estádios críticos da ontogenia dos frutos como, por exemplo, durante a iniciação do primórdio floral (Rajagopal et al., 1996). Plantas de coqueiro da variedade gigante (*C. nucifera* L. ‘*typica’*) submetidas à deficiência hídrica tiveram o potencial hídrico foliar (Ψ) reduzido a -1,4 MPa e mostraram uma queda de 19 e 29%

na produção de matéria seca vegetativa e reprodutiva, respectivamente (Rajagopal et al., 1989). No Brasil, existem relatos de aumentos da ordem de 200% na produção de frutos por planta quando se utiliza a irrigação (Camboim, 2000).

O perfil de respostas do coqueiro ao déficit hídrico está, de uma maneira geral, bem definido (Rajagopal e Kasturi Bai, 2002). Inúmeros trabalhos mostraram redução da condutância estomática ao vapor de água (gs) e do Ψ_{wf}, com reflexos negativos sobre a taxa de assimilação líquida de CO_2 (A) e a transpiração (E) como efeitos imediatos da deficiência hídrica, seja por depleção da água do solo ou por uma elevada demanda evaporativa (Reppellin et al., 1994; 1997; Passos et al., 1999; Prado et al., 2001; Gomes et al., 2002). Voleti et al. (1990) observaram uma relação inversa entre o teor relativo de água e o acúmulo de prolina em diversos genótipos de coqueiro submetidos a estresse e concluíram que o teor de prolina não deve ser associado à tolerância à seca. Por outro lado, Jayasekara et al. (1993) observaram acúmulo de prolina em genótipos de coqueiro tolerantes à deficiência hídrica durante os meses secos. Embora o ajuste osmótico tenha sido definido como um importante mecanismo de tolerância ao déficit hídrico em coqueiro (Kasturi Bai e Rajagopal, 2000), o papel específico da prolina permanece controverso.

Dois ecotipos do cultivar anão-verde do Brasil, cultivados há muito tempo em regiões contrastantes com relação ao clima, têm sido bastante utilizados para a formação de novos plantios: Anão-Verde de Una (UGD) e Anão-Verde de Jiqui (JGD). Espera-se, portanto, que esses ecotipos respondam de maneira diferente à deficiência hídrica. Inúmeros estudos têm mostrado a ocorrência de variação significativa entre os diversos genótipos de coqueiro na manifestação de alguns mecanismos de tolerância, o que, muitas vezes, tem permitido a classificação dos genótipos em tolerantes e não-tolerantes (Rajagopal et al., 1990; Reppellin et al., 1994; Passos et al., 1999; Prado et al., 2001). Por apresentar, entre outras características, maior freqüência estomática e elevada taxa transpiratória, o coqueiro anão está sujeito a uma maior perda de água se comparado ao gigante (Rajagopal et al., 1990). Entre os anões destaca-se o Amarelo da Malásia, que apresenta características vantajosas como melhor capacidade de regulação estomática e um sistema radicular mais desenvolvido, o que lhe permite responder de maneira mais adequada às variações do potencial de água no solo (Passos et al., 1999). Esses autores realizaram um estudo comparativo importante quanto à capacidade de regulação estomática frente à seca entre três genótipos de anão em condições de campo, com plantas adultas. Entretanto, não se encontrou nenhum estudo comparando os anões verdes cultivados no Brasil, quanto aos mecanismos de tolerância à deficiência hídrica identificados em outros genótipos (Rajagopal e Kasturi Bai, 2002). Em alguns trabalhos com o cultivar anão-verde, a redução na taxa fotossintética tem sido explicada pela redução na condutância estomática (Passos et al., 1999; Gomes et al., 2002). Entretanto, outros efeitos como, por exemplo, ocorrência de danos ao sistema de transporte de elétrons dos cloroplastos, fotoinibição, alterações na capacidade fotossintética por limitações não-estomáticas, além de ajuste osmótico e participação do ácido abscísico como sinalizador para a regulação estomática, têm sido pouco estudados.

Dessa forma, objetivou-se avaliar características fisiológicas e bioquímicas associadas à tolerância à deficiência hídrica em dois ecotipos de coqueiro anão-verde submetidos a três ciclos sucessivos de seca/recuperação. Especificamente, buscou-se (1) avaliar os efeitos do déficit hídrico e posterior recuperação no desenvolvimento inicial, nas trocas gasosas, nas relações hídricas, na integridade do sistema fotossintético de transporte de elétrons, (2) avaliar a ocorrência de ajuste osmótico, procurando definir o papel da prolina, além da participação do ácido abscísico no controle estomático do coqueiro sob deficiência hídrica e (3) determinar se os dois ecotipos respondem de maneira diferente à deficiência hídrica, procurando identificar características que possam ser úteis aos programas de melhoramento genético com vistas à obtenção de materiais mais eficientes com relação ao uso da água. Adicionalmente, objetivou-se comparar três modelos empíricos, com relação ao seu desempenho quantitativo e qualitativo para descrever a resposta da fotossíntese à irradiância.

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CAPÍTULO 1

Changes in photosynthesis, water relations and levels of abscisic acid and proline in leaves of Brazilian Green Dwarf coconut induced by repeated cycles of water deficit.

Abstract The area cultivated with dwarf coconut palm (*Cocos nucifera* var. *nana* L.) in Brazil has increased in the last years mainly for the production of tender coconut water in both the atlantic coastal strip and some inland areas. With the objective of evaluating the impact of repeated cycles of water stress on some physiological and biochemical traits of young plants of two Brazilian Green Dwarf coconut ecotypes (Brazilian Green Dwarf from Una, Bahia, UGD, and from Jiqui, Rio Grande do Norte, JGD), a pot experiment was conducted under greenhouse conditions. Four main hypotheses were tested: (1) the photosynthetic gas exchange is severely affected by the drought stress but the effect is progressively small after successive drying/recovery cycles, (2) the coconut stomata aperture is regulated by the accumulation of abscisic acid regulates under mild water shortage, (3) the osmotic adjustment in coconut is independent of proline accumulation and (4) the two ecotypes show distinct pattern of response to the drying/recovery cycles. The plants were submitted to three drought cycles consisting of suspension of irrigation until the net photosynthetic rate (*A*) approached zero and rewatering until recovery of the assimilation rates to, at least, 85% of the control. *A*, stomatal conductance (*gs*) and transpiration (*E*) were negligible when the pre-dawn leaf water potential (Ψ_{PD}) reached about -1.2 MPa, being observed reduction in *gs* and *E* before significant decreases in *A*. It was observed that *gs* = 0.04 mol m^{-2} s^{-1} and Ψ_{PD} = -1.0 MPa were the threshold values bellow which the intercellular CO_2 concentration (*Ci*) increased and the intrinsic water use efficiency (*A*/*gs*) decreased, indicating that non-stomatal factors are limiting *A*. Decreases in *gs* and increases in leaflet abscisic acid concentration ([ABA]) were observed before significant changes in the leaf water status, indicating that *gs* was controlled by ABA under mild water stress.

In addition, *A*/*gs* was improved at mild water deficit without significant impairment of *A*. Green dwarf coconut palm showed osmotic adjustment in response to water deficit as well as significant impairment of growth. Expressive accumulation of proline in leaflets was observed in the two ecotypes, which was more related to protective rather than to osmoregulatory functions. Slight differences among the ecotypes with respect to the response to treatments were observed, the JGD showing higher *A*/*gs* after repeated cycles of water stress, as well as, higher proline accumulation capacity.

Keywords: Abscisic acid; *Cocos nucifera*; Drought tolerance; Gas exchange; Osmotic adjustment; Osmotic potential; Water relations.

Resumo No Brasil, a área cultivada com coqueiros anões (*Cocos nucifera* var. *nana* L.) para a produção de água-de-coco tem aumentado nos últimos anos, tanto nas áreas litorâneas quanto no interior. Com o objetivo de avaliar o impacto de ciclos repetidos de deficiência hídrica sobre algumas características fisiológicas e bioquímicas de plantas jovens de dois ecotipos de coqueiro Anão-Verde do Brasil (procedente de Una, Bahia, UGD e de Jiqui, Rio Grande do Norte, JGD), conduziu-se um experimento em vasos sob condições de casa de vegetação. Quatro principais hipóteses foram testadas: (1) as trocas gasosas são severamente afetadas pela deficiência hídrica, mas os efeitos são progressivamente menores depois de repetidos ciclos de desidratação; (2) a abertura estomática é regulada pelo acúmulo de ácido abscísico nas folhas sob condições de deficiência hídrica moderada; (3) o ajustamento osmótico em plantas estressadas está relacionado ao acúmulo de prolina nas folhas e (4) os dois ecotipos diferem quanto ao perfil de resposta aos ciclos de desidratação. As plantas foram submetidas a três ciclos de desidratação que consistiram em suspender a irrigação até a taxa fotossintética líquida (*A*) atingir um valor próximo de zero, retomando as irrigações em seguida até recuperação de *A* a, no mínimo, 85% do controle. *A*, condutância estomática (*gs*) e transpiração (*E*) atingiram valores próximos a zero quando o potencial hídrico foliar medido antes do amanhecer (Ψ_{PD}) atingiu -1,2 MPa, sendo que *gs* e *E* foram reduzidos antes de decréscimos significativos em *A*. Observou-se que *gs* = 0,04 mol m^{-2} s^{-1} e Ψ_{PD} = -1,0 MPa foram os valores limite abaixo dos quais a concentração de CO_2 nos espaços intercelulares (*Ci*) aumentou e a eficiência intrínseca de uso da água (*A*/*gs*) decresceu, indicando a ocorrência de limitações não-estomáticas à fotossíntese. Alem disso, g*s* decresceu e a concentração foliar de ácido abscísico (ABA) aumentou expressivamente antes de alterações significativas no estado hídrico da folha, indicando que *gs* é controlada por ABA sob estresse hídrico moderado. Adicionalmente, *A*/*gs* aumentou expressivamente sob estresse hídrico moderado antes que decréscimos significativos em *A* fossem observados. O coqueiro Anão-Verde apresentou ajuste osmótico de 0,05 a 0,2 MPa em resposta ao défice hídrico, bem como redução significativa no crescimento. Observou-se acúmulo significativo de prolina nas folhas das plantas estressadas dos dois ecotipos, o qual foi relacionado mais a funções protetoras do que osmoreguladoras. Diferenças discretas entre os dois ecotipos foram observadas, o JGD mostrado maior *A*/*gs* após repetidos ciclos de deficiência hídrica, bem como maior capacidade de acúmulo de prolina.

1. Introduction

Water availability is the environmental factor that most strongly affects plant growth and yield worldwide (Nemani *et al.* 2003). In many cases, increased irrigation inputs are not a viable option because the water resources are either scarce or too expensive. In a world limited by supplies of freshwater, the trend is towards greater restrictions on agricultural water use. Moreover, there have been considerable progress in the understanding of the mechanisms that allow plants to survive severe water stress, although most of then may well be of limited interest in most agricultural contexts, because they address the problem of survival rather than that of production (Tardieu, 2005).

Plants can sense the water availability around the roots and respond by sending chemicals signals to the shoot to elicit several adaptative responses, such decrease in leaf expansion growth and stomatal closure (Wilkinson and Davies, 2002). Abscisic acid (ABA) has been suggested as a major signaling molecule involved in the response of plants to drought stress. As the soil dries, ABA is produced at the root tips and transported via the transpiration stream reaching the leaf where it reduces stomatal conductance (Zhang and Outlaw Jr., 2001). It is well known that these responses can occur without any change in shoot water relations (Wilkinson and Davies, 2002). Besides the control of stomatal aperture, ABA is thought to play an important role in increasing the influx of ions across the membranes in the roots as well as, in the synthesis and accumulation of osmotically active solutes, such amino acids (particularly proline), sugars, inorganic ions (LaRosa et al., 1987).

It has been suggested that the accumulation of some compounds such as sugars, amino acids, alcohols and quaternary ammonium is an important feature of overcoming environmental stresses (Morgan, 1984). Those so called compatible osmolytes are thought to lower water activity and raise the osmotic potential of the cell, so that turgor and turgor-related processes may be maintained during drought episodes. Moreover, the osmolyte may act as osmoprotectants, stabilizing macromolecular structures and/or subcellular membranes (Morgan, 1984).

The area cultivated with dwarf coconut palm (*Cocos nucifera* var. *nana* L.) in Brazil has increased in the last years mainly for the production of tender coconut water in both the atlantic coastal strip and some inland areas. The interest in the cultivation of dwarf coconut has increased the demand of information concerning the growth, development and yield under different ecological conditions. In this way, ecophysiological approach deserves special attention, since it permits to unravel the responses of many physiological traits at leaf and whole plant levels due to environmental changes (Rajagopal and Kasturi Bai, 1999; Passos et al., 1999; Prado et al., 2001; Gomes et al., 2002). The research on drought tolerance of coconut brought out some important findings such the impact of irrigation levels and whether parameters on the physiology and biochemistry of this crop (Reppelin et al., 1997; Rajagopal and Kasturi Bai, 1999; 2002). Higher dry matter production on drought tolerant types has been implied as result of an efficient stomatal regulatory mechanism, (Kasturi Bai et al., 1996), of leaflet anatomical adaptations (Naresh Kumar et al., 2000), and also to some degree of osmotic adjustment to overcome mild stress conditions (Kasturi Bai and Rajagopal, 2000).

However, traits such as drought-induced proline accumulation (and its link to osmotic adjustment) and the role of abscisic acid (ABA) as a root-to-shoot non-hydraulic signal of soil drought still are under debate, since the works of Kasturi Bai and Rajagopal (2000) and of Reppelin et al. (1997), respectively. The aim of this study was to evaluate the impact of repeated cycles of water stress on growth, dry matter accumulation, water relations and photosynthetic gas exchange in two Brazilian Green Dwarf coconut ecotypes submitted to three successive cycles of water deficit.

2. Material and Methods

2.1. Plant Material and growing conditions

A pot experiment was conducted at the Plant Growth Unit of the Federal University of Viçosa, Brazil (20º 45’S, 42º 52’W, 648masl) with two ecotypes of the Brazilian Green Dwarf cultivar: Brazilian Green Dwarf from Una (UGD) is cultivated at the southeast region of Bahia State (15º15'S, 39º05'W, 105 masl), which is characterized by hot and humid climate, high indexes of rain fall (> 1500 mm year^{-1}), air relative humidity above 80% and mean temperature between 24 and 27ºC (Almeida e Santana, 1999); Brazilian Green Dwarf from Jiqui (JGD) is cultivated in the Rio Grande do Norte State (05º47'S, 35º12'W, 30 masl), a contrasting semi-arid environment characterized by high temperatures (maximum of 33ºC and minimum of 23ºC), low pluviometric precipitation (< 650 mm year^{-1}) and average air relative humidity of 69% (Carmo Filho *et al.* 1991). The ecotypes UGD and JGD are morphologically similar, being observed slight differences between then in volume of tender coconut water (personal communication).

The plants were grown in pots of 150 L filled with a soil/sand mixture (1:2), under greenhouse conditions. The daily average values of air temperature and solar irradiance inside the greenhouse were measured with a non-linear thermistor and a LI-200SA pyranometer sensor, respectively. Both sensor were connected to a datalogger LI-1200 Data Set Recorder (LI-COR, Inc. USA). Relative humidity was measured with a "harp" of human hair sensor type of a thermohygrograph calibrated weekly against a ventilated wet bulb psycrometer. The microclimatic data of each drying/recovery cycle are shown in Table 1. The photosynthetic active radiation, measured with a quantum sensor LI-190SA (LI-COR, Inc. USA), ranged from 200 to 1200 μmol m^{-2} s^{-1} during the sampling period. The plants were irrigated daily to maintain a soil water content close to field capacity (-0.002 MPa). The soil water potential at field capacity was estimated after the construction of a soil water retention curve, following the moisture equivalent method (Ruiz et al. 2003). All essential nutrients were supplied, following the leaf and soil analyzes and the requirements of the specie (Magat, 2003).

2.2. Drought/recovery treatments

After 12 months, the drought treatment was initiated by suspension of irrigation until the net photosynthetic rate (*A*) approached zero (soil water potential -0.52 MPa), which was considered the point of maximum stress (PMS). Then that so called water stressed (WS) plants were rewatered until recovery of the assimilation rates to values > 85% in relation to the well watered (WW) control plants. This procedure was repeated two times, giving a total of 3 consecutive drying/recovery cycles. The drought period lasted 44, 35 and 35 days for cycles 1, 2 and 3, respectively.

2.3. Leaflet gas exchange measurements

Net photosynthetic rate (*A*), stomatal conductance to water vapour (*gs*) and transpiration rate (*E*) were measured weekly always from 8:30 to 11:30 h a.m. with a Portable Photosynthesis System LI-6400 (LI-COR Biosciences Inc., Nebraska, USA). Irradiance (*PPFD*), atmospheric CO_2 concentration (*Ca*) and leaf temperature were fixed at, respectively, 1200 µmol photons m^{-2} s^{-1}, 380 µmol mol^{-1}, and 26ºC using the equipment's accessories. The readings were stable in 2-3 min (coefficient of variation < 1%) when they were saved by the LI-6400. The intercellular CO_2 concentration (*Ci*) was calculated by the equipment from the values of *A*, *gs* and *E* (von Caemmerer and Farquhar, 1981). Leaflets of the middle of rank two leaves (counting from the top taking the spindle as zero) were used for the measurements. The measurements were also performed at the fourth and eighth days after rewatering the plants.

2.4. Leaflet water potential and osmotic adjustment

Pre-dawn leaflet water potential (Ψ_{PD}) was measured weekly at 4:30 am in one leaflet per plant of four plants per treatment, with a pressure chamber Skye SKPM 1400 (Skye Instruments LTD., UK). All measurements were done on opposite leaflets of those used for gas exchange measurements, following the methodology described by Milburn and Zimmermann (1977) with modifications. Briefly, after detaching the leaflet, the base of the lamina was trimmed with a sharp knife to free the cylindrical midrib, the leaflet was rolled (keeping the adaxial surface outermost to avoid breaking the rib) and sealed into the pressure chamber. The pressure was increased slowly (0.02 MPa s^{-1}) with compressed nitrogen until the sap was visible at the exposed base of the midrib, characterizing the balancing pressure.

Osmotic potential was measured at the points of maximum stress and after eight days of recovery in each cycle. Samples of mature leaf blades stored in plastic bags at -20°C were thawed and pressed in a hand press. Then the liquid obtained was centrifuged at 2500 *g* for 20 min and the supernatant used for determination of osmotic potential (*s*) in four replicates for each treatment of each ecotype. Determination of Ψ_S was performed by a cryoscopic method using a microosmometer (Osmette 2007, Precision System, Inc, USA), according to Boyer (1995). To avoid the effects of passive concentration associated to water loss (Babu et al., 1999), the values of Ψ_S (stressed and control) were multiplied by a factor based on the tissue dry weight which referenced osmotic potential to a common water content of 70% of fresh weight (Alves and Setter, 2004). Osmotic adjustment (OA) was calculated as the difference in corrected osmotic potential between control and stressed plants.

2.5. Proline and ABA determinations

Simultaneously with the gas exchange measurements, samples of leaflet tissue were collected, immediately frozen in liquid nitrogen and stored at -80 ºC until the moment of the analyses. Proline concentration was determined in 80% ethanol extracts of fresh leaf tissue by the nyhnidrin method of Bates *et al*. (1973). ABA concentration in samples of frozen fresh leaflet tissue was determined using a radioimmunoassay (RIA) according to Quarrie et al. (1988), using the monoclonal antibody (MAC252) which is specific for (+)-ABA (Barrieu and Simonneau, 2000). Standard ABA samples were included in each assay for the construction of a standard curve. The validation of RIA for use with unpurified leaflet extracts was checked by a spike dilution test for non-specific interferences, after Jones (1987).

2.6. Growth and dry mass measurements

Girth size measured at collar and number of leaves were measured monthly during the experiment. At the end of the experiment, total leaf area and dry mass production in root and aerial part (shoots and leaves) were estimated by allometric models previously adjusted for this purpose. Data of maximum length and width of the palmed part of leaf rank four and the girth at collar were used as allometric coefficients.

2.7. Statistics

For each ecotype, 20 plants (12 stressed and 8 controls) were organized in a completely randomized design. The data of all measured and/or calculated variables were submitted to three-factorial analyze of variance (ANOVA), with treatment (drought and control), ecotype (UGD and JGD) and cycle as main factors. The data were pooled for the subsequent analyses when non-significant effect of ecotype was detected by the ANOVA. The Tukey’s test at 5% of probability was used for discriminating among the means of the variables. The statistical procedures were performed using the Statistica 6.0 (Statsoft Inc., USA) and the CurveExpert 1.3. (Microsoft Corporation).

3. Results

Except the relative humidity, all the microclimatic variables inside the greenhouse were reduced in the cycle 3, as compared to cycles 1 and 2 (Table 1).

Table 1. Average (s.e.) daily values of maximum (Tmax), minimum (Tmin) and mean (Tmean) air temperature, solar irradiance (SI) and relative humidity measured inside the greenhouse, during the drying/recovery cycles. For each variable, means followed by the same letter are not different by the tukey's test (P 0.05)

	Cycle 1	Cycle 2	Cycle 3
Tmax (ºC)	30.8 (0.5)^{a}	31.4 (0.3)^{a}	29.2 (0.4)^{b}
Tmin (ºC)	19.4 (0.2)^{a}	19.2 (0.2)^{a}	17.3 (0.2)^{b}
Tmean (ºC)	23.7 (0.2)^{a}	23.8 (0.2)^{a}	21.8 (0.2)^{b}
SI (MJ m^{-2})	7.5 (0.4)^{a}	7.0 (0.2)^{a}	4.3 (0.2)^{b}
RH (%)	76.7 (1.2)^{a}	73.7 (0.8)^{a}	73.5 (1.4)^{a}

3.1. Leaf water potential and gas exchange

In well-watered plants, pre-dawn leaf water potential (Ψ_{PD}) fluctuated around an average value of -0.1 MPa during the experimental period (Figure 1). With an increasing water stress, Ψ_{PD} decreased significantly 8 days after the imposition of stress (DAIS), reaching -1.2 MPa at the point of maximum stress without significant differences between cycles (Figure 1). Upon rewatering, a rapid and full recovery of Ψ_{PD} was observed.

A, *gs* and *E* of well-watered plants increased in WW plants during the period of measurements, respectively, from 11 to 15 µmol m^{-2} s^{-1}, from 0.24 to 0.43 mol m^{-2} s^{-1} and from 2.5 to 3.5 mmol m^{-2} s^{-1} (Figure 1). That increase could be related to the reduction in leaf temperature following the decreasing trend of air temperature and solar radiation inside the greenhouse from the beginning to the end of the experimental period (Table 1). In WS plants *A*, *gs* and *E* started to diverge from the well-watered controls after 16 (Ψ_{PD} = -0.56 MPa), 8 (Ψ_{PD} = -0.27 MPa) and 8 days after the imposition of stress (DAIS), respectively (Figure 1). At the PMS (*A* 0 mol m^{-2} s^{-1}), *gs* and *E* in WS plants were 3 and 6% of their controls, respectively, without significant differences between cycles. Upon rewatering for eight days, *A* and *E* recovered to values > 85% in relation to their controls irrespective of the cycle, whereas *gs* was 84, 69 and 67% in relation to WW plants in the cycles 1, 2 and 3, respectively (Figure 1).

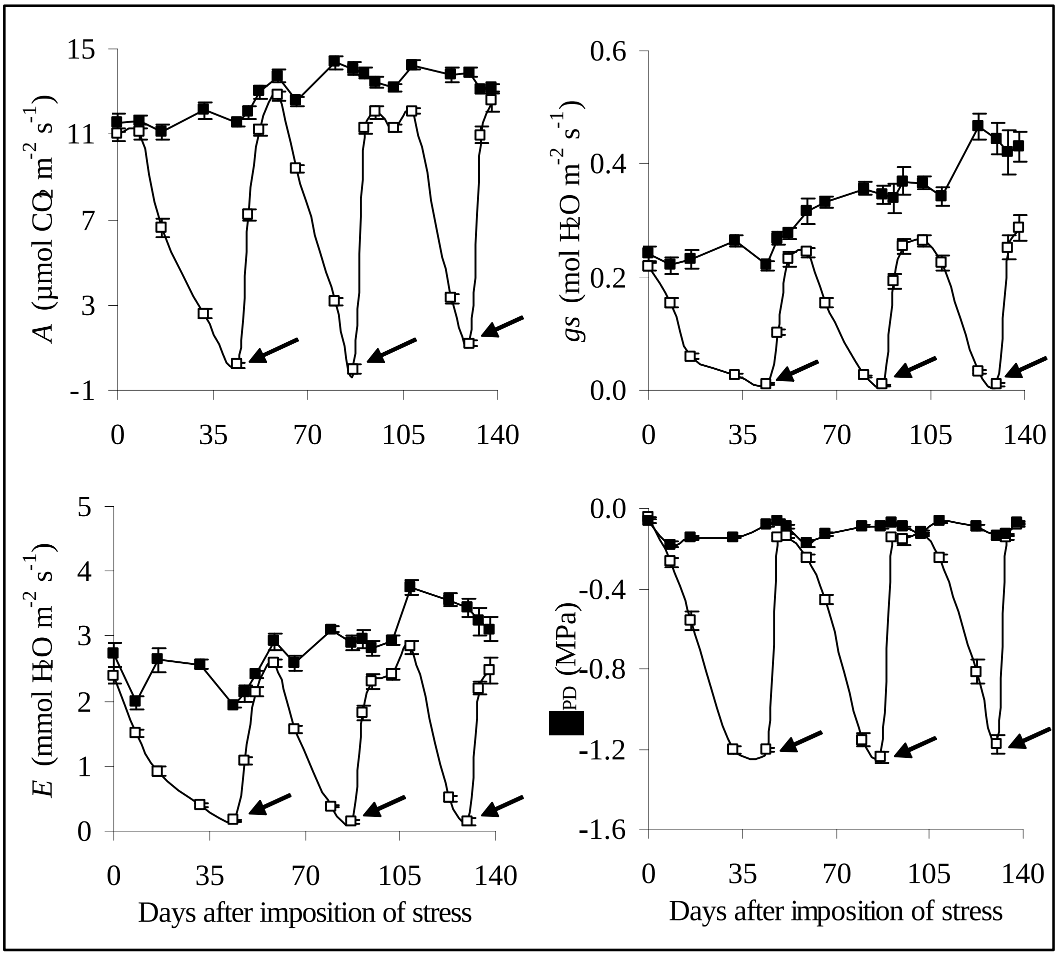

Figure 1. Photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*) and pre-dawn leaf water potential (Ψ_{PD}) of well-watered (solid) and water stressed (open) plants of Brazilian Green Dwarf coconut. Values are means (n = 6 – 24) of the two ecotypes and error bars are the standard error of means (s.e.). The arrows indicate the moments of rewatering.

After the onset of drought stress, intrinsic water use efficiency (*A*/*gs*) increased and the ratio of internal to atmospheric CO_2 concentration (*Ci*/*Ca*) decreased and reached a peak just before the point of maximum stress (Figure 2). The increase in *A*/*gs* and the decrease in *Ci*/*Ca* were more pronounced in JGD than in UGD at the third cycle. When *A*/*gs* was plotted against *gs* (Figure 3a) and Ψ_{PD} (Figure 3b), it was possible to estimate a threshold value of *gs* (0.04 mol m^{-2} s^{-1}) and Ψ_{PD} (-1.0 MPa) below which *A*/*gs* decreased sharply.

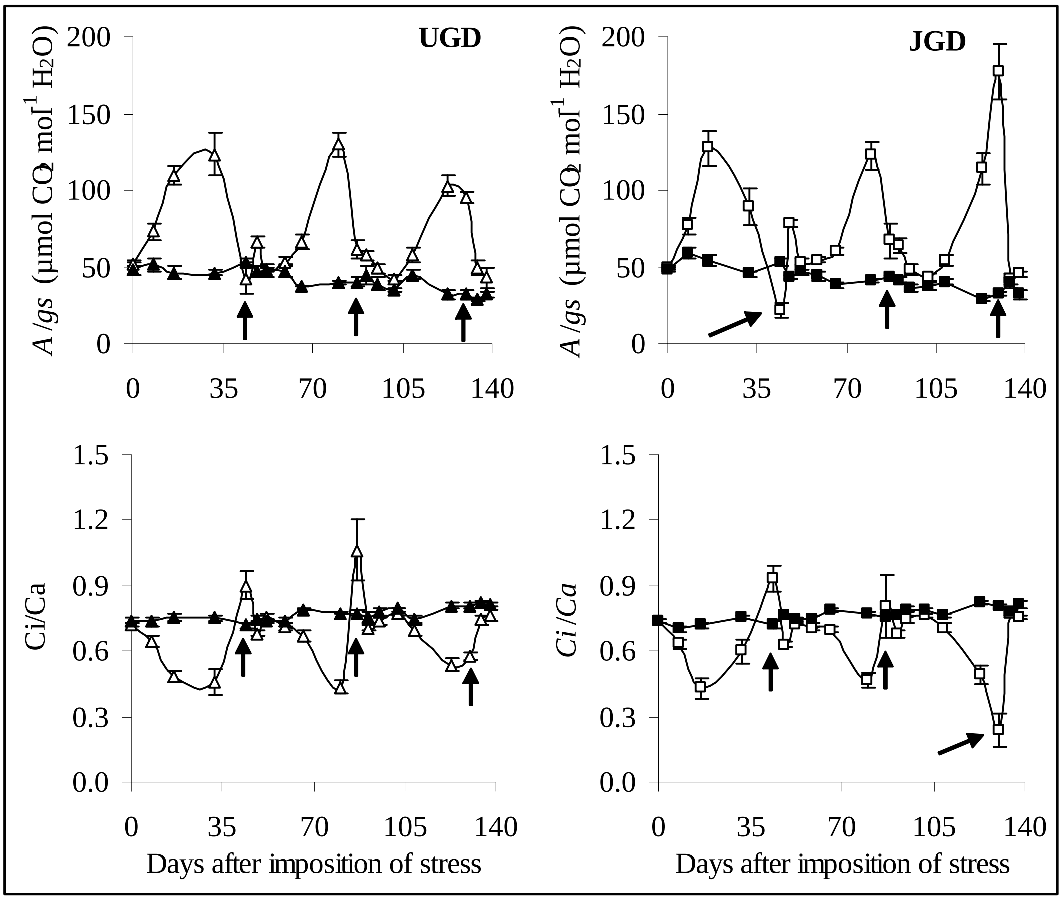

Figure 2. Intrinsic water use efficiency (*A*/*gs*) and the ratio of intercellular to atmospheric CO_2 concentration (*Ci*/*Ca*) of well-watered (solid) and water stressed (open) plants of Brazilian Green Dwarf coconut from Una (UGD) and from Jiqui (JGD). Values are means (n = 4 – 12) and the error bars are the s.e. The arrows indicate the moments of rewatering.

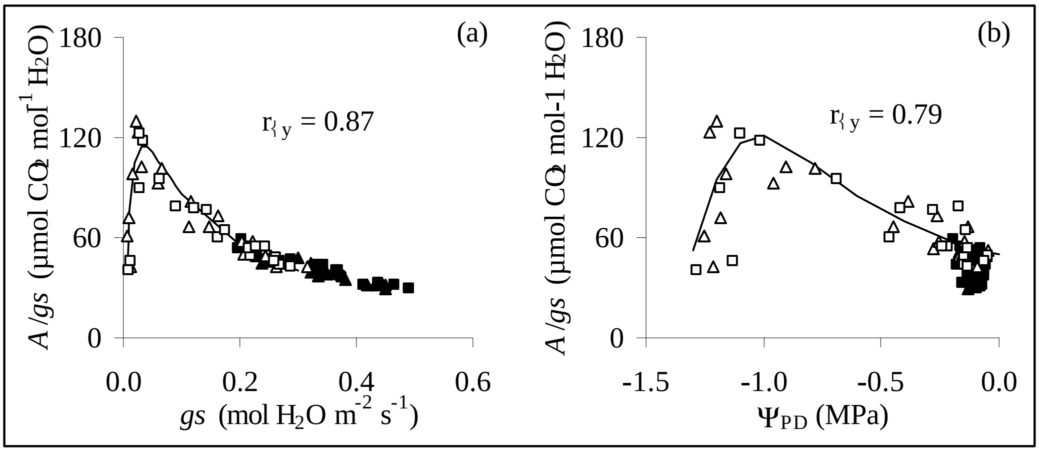

Figure 3. Intrinsic water use efficiency (*A*/*gs*) expressed as a function of stomatal conductance (*gs*, a) and pre-dawn leaf water potential (Ψ_{PD}, b) of well-watered (solid) and water stressed (open) plants of Brazilian Green Dwarf coconut from Una (UGD, triangles) and from Jiqui (JGD, squares). Values are means (n = 4 – 12) and the error bars are the s.e.. The lines represent the rational model.

3.2. *Abscisic acid concentration ([ABA])*

In WW plants, [ABA] ranged from 0.5 to 2.0 nmol g^{-1} DW during the sampling period (Figure 4). Drought stress led to increases in [ABA], which were detected after 16 DAIS in the two ecotypes. In WS plants of UGD, the values of [ABA] increased 2.8, 2.5 and 4.0 times in relation of control plants, whereas increased 3.6, 2.4 and 2.6 times in JGD at the cycles 1, 2 and 3, respectively (Figure 4).

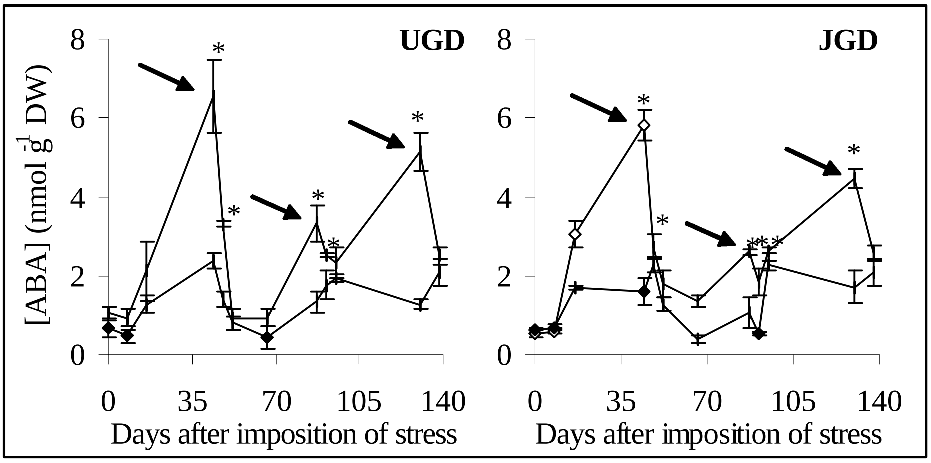

Figure 4. Abscisic acid concentration ([ABA]) in leaflets of well-watered (solid) and water stressed (open) plants of Brazilian Green Dwarf coconut from Una (UGD) and from Jiqui (JGD). Values are means (n = 4) and the error bars are the s.e.. * Represent significant ($P \leq 0.05$) differences by the *F* test. The arrows indicate rewatering.

3.3. Osmotic adjustment and proline accumulation

Corrected values of osmotic potential (Ψ_S) were significantly reduced (more negative) in WS plants of UGD at PMS and recovery phases of cycle 1 and at PMS of cycle 3 (Table 2). In JGD, the reduction was significant at the PMS of all the three cycles and at the recovery phase of cycle 2. In addition, Ψ_S decreased after the imposition of successive cycles in both WW and WS plants of the two ecotypes. The osmotic adjustment at the point of maximum stress ($\Delta\Psi_S = \Psi_{S(control)} - \Psi_{S(stress)}$) varied from 0.05 to 0.24 MPa in UGD and from 0.10 to 0.15 MPa in JGD (Table 2). Moreover, the osmotic adjustment in leaflets of UGD was more than twice the values of JGD at the cycle 1. At the end of experiment, both ecotypes showed and equivalent osmotic adjustment (Table 2).

Table 2. Osmotic potential (Ψ_S) in plants of Brazilian Green Dwarf coconut from Una (UGD) and from Jiqui (JGD). Osmotic adjustment (OA) was calculated as the differences in Ψ_S between well watered and water stressed plants. Values are mean (± se, n = 4). Lower case letters indicate significant differences between treatments in each line and capital letters indicate significant differences among ecotypes within each treatment by the *F* test ($P \leq 0.05$)

			Ψ_S (MPa)		
Ecotype	Cycle		Water stressed	Well-watered	OA
UGD	1	PMS	-1.85 ± 0.03 ^{aA}	-1.63 ± 0.04 ^{bA}	0.22
		REC	-1.71 ± 0.14 ^{aA}	-1.47 ± 0.03 ^{bA}	0.24
	2	PMS	-1.51 ± 0.01 ^{aA}	-1.47 ± 0.03 ^{aB}	0.05
		REC	-1.84 ± 0.04 ^{aA}	-1.70 ± 0.11 ^{aA}	0.14
	3	PMS	-1.46 ± 0.05 ^{aA}	-1.30 ± 0.05 ^{bA}	0.16
		REC	-1.44 ± 0.03 ^{aA}	-1.35 ± 0.08 ^{aA}	0.09
JGD	1	PMS	-1.74 ± 0.01 ^{aA}	-1.64 ± 0.02 ^{bA}	0.10
		REC	-1.72 ± 0.01 ^{aA}	-1.61 ± 0.04 ^{aA}	0.11
	2	PMS	-1.48 ± 0.02 ^{aA}	-1.37 ± 0.04 ^{bA}	0.12
		REC	-1.68 ± 0.03 ^{aB}	-1.51 ± 0.05 ^{bA}	0.17
	3	PMS	-1.34 ± 0.02 ^{aB}	-1.18 ± 0.08 ^{bA}	0.15
		REC	-1.44 ± 0.09 ^{aA}	-1.29 ± 0.04 ^{aA}	0.15

The consecutive drought/recovery cycles led to significant increases in leaflet proline concentration of the two ecotypes, which were less pronounced from the first to the third cycles (Figure 5). Proline content in control plants was about 1.5 µmol g^{-1} DW on average. At the point of maximum stress of cycles 1, 2 and 3 proline concentration in leaflets of WS plants increased, respectively 1.9, 1.9 and 1.5 times in UGD and 2.1, 1.5 and 1.5 times in JGD in relation to their controls. Except in JGD at the cycle 3, the concentration of proline recovered to the control levels after rewatering for eight days.

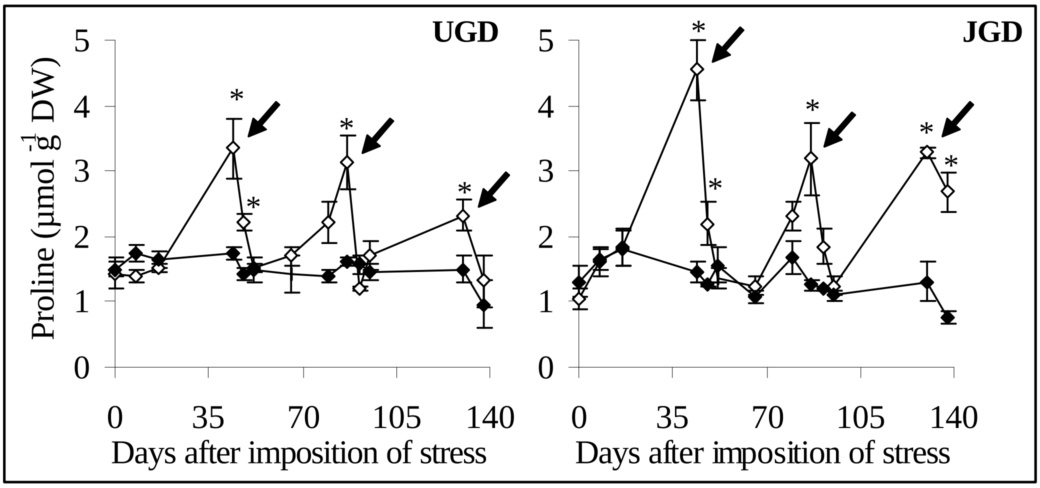

Figure 5. Proline concentration in leaflets of well-watered (solid) and water stressed (open) plants of green dwarf coconut from Una (UGD) and from Jiqui (JGD). Values are means (n = 4) and error bars are the s.e.. * Represent significant ($P \leq 0.05$) differences by the *F* test. The arrows indicate the moments of rewatering.

3.4. Control of stomatal behavior

The relationship between *A* and *gs* was well represented by an exponential function (Figure 6a), which demonstrated that (1) only small non-significant decreases in *A* (9%) were observed when *gs* decreased 44%, from 0.5 to about 0.28 mol m^{-2} s^{-1} and (2) the relationship between the two variables became linear only at *gs* < 0.05 mol m^{-2} s^{-1}. The small decrease of *A* at high *gs* may be attributed to the small reductions in *Ci* within this range of *gs* (Figure 6c). In addition, the same decrease of *A* (9%) occurred when Ψ_{PD} was reduced from -0.11MPa (the average value of WW plants) to about -0.27 MPa (Figure 6b).

gs decreased from 0.5 to 0.2 mol m^{-2} s^{-1} without great changes in Ψ_{PD} (Figure 6d). The relationship between *gs* and Ψ_{PD} (Figure 6d) and between *gs* and [ABA] (Figure 6e) were curvilinear and linear, respectively, when *gs* decreased from 0.2 to 0.05 mol m^{-2} s^{-1}. A truly linear relationship between *g*s and Ψ_{PD} was observed only when Ψ_{PD} was lower than -0.5MPa (Figure 6d). [ABA] increased exponentially with reducing Ψ_{PD}, showing slow initial increase in $\Psi_{PD} > -0.5$MPa and a sharp increase thereafter (Figure 6f).

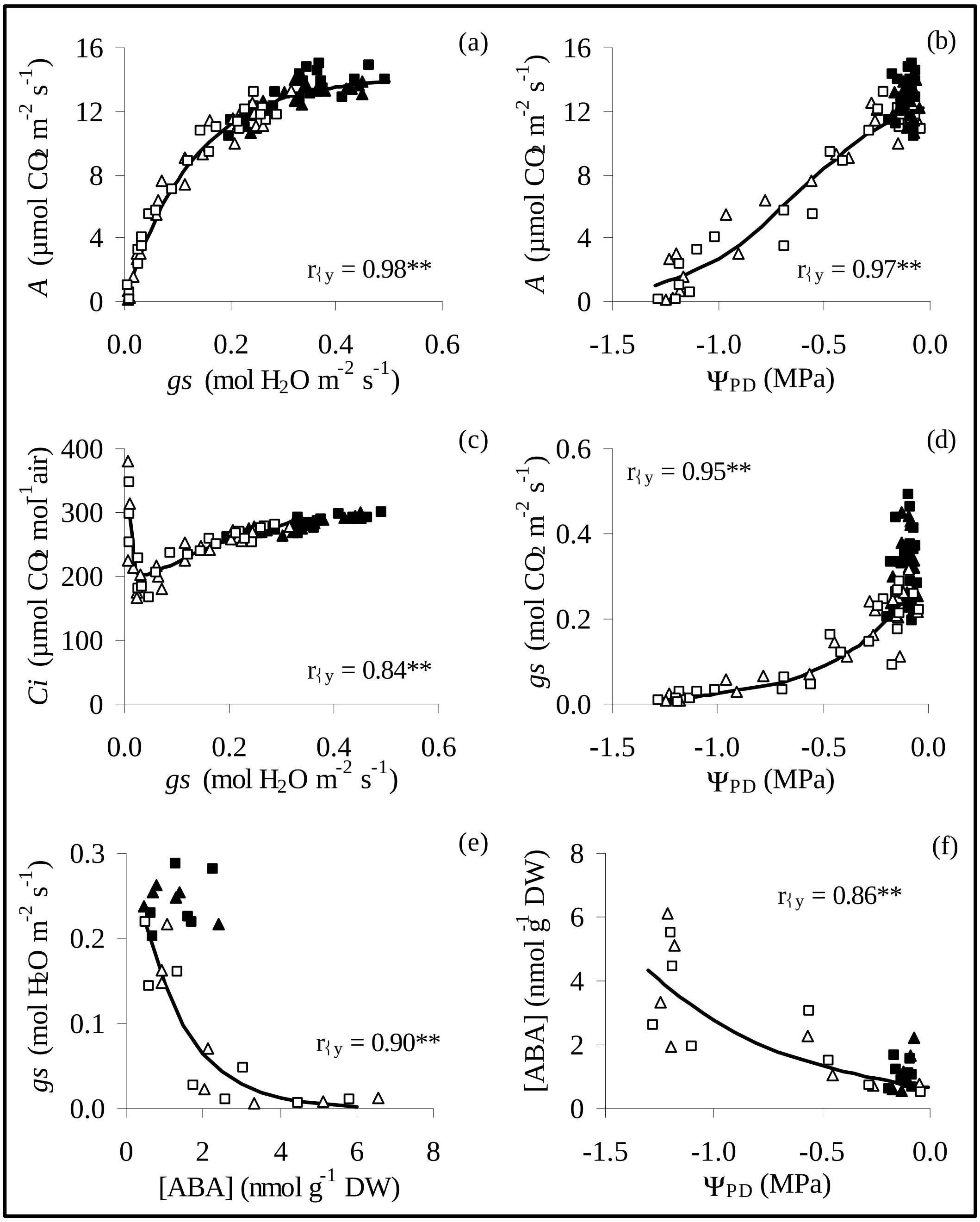

Figure 6. Photosynthesis (*A*) as a function of stomatal conductance (*gs*, a) and of pre-dawn leaf water potential (Ψ_{PD}, b); intercellular CO_2 concentration (*Ci*) as a function of *gs* (c); *gs* as a function of pre-dawn leaf water potential (Ψ_{PD}, d) and of leaflet abscisic acid concentration ([ABA], e) and [ABA] as a function of Ψ_{PD} (f) in well-watered (solid) and water stressed (open) plants of Brazilian Green Dwarf coconut from Una (triangles) and from Jiqui (squares). Values are means (n = 4 - 12). The lines represent the exponential (a, d, e, f), the rational (b) and the modified Hoerl (c) models.

3.5. Growth and dry mass production

Girth size and number of leaves measured during the experimental period are shown in the figure 7. Both variables were negatively affected just after the onset of the drought treatments, the number of leaves being reduced earlier than girth size. Drought stress also led to severe reductions in total leaf area (27%), total dry matter (52%), and root to shoot ratio (25%) (Table 3). There were no significant effects of drought treatments on specific leaf area.

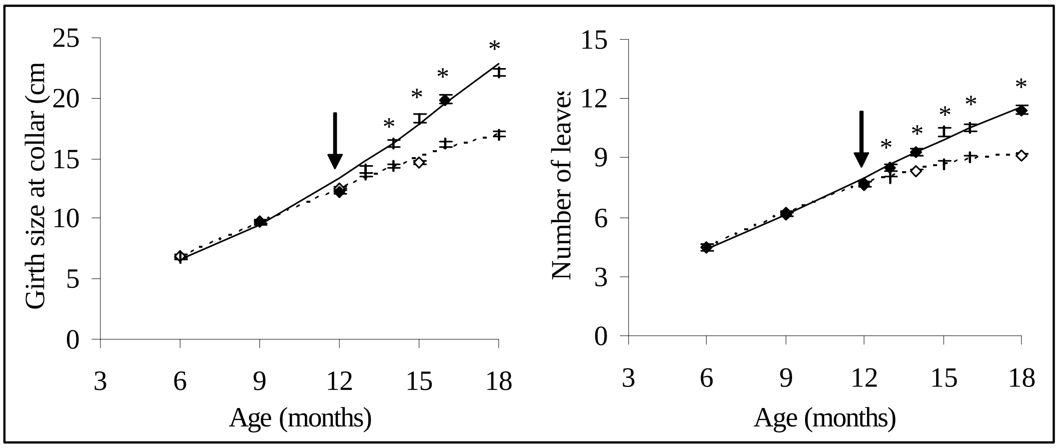

Figure 7. Girth size at collar and number of leaves in the crown of Brazilian Green Dwarf coconut, measured during the experimental period in well-watered (solid) and water stressed (open) plants. The values are mean (n = 16-24) of the two ecotypes and the error bars are the s.e..* Represent significant ($P \leq 0.05$) differences by the *F* test. The arrows indicate the beginning of the drought treatment. The lines represent the logistic model ($r\{_y > 0.99$).

Table 3. Total (LA) and specific (SLA) leaf area, total dry mass (TDM) and root to shoot ratio (R/S) in well-watered (WW, n=10) and water stressed (WS, n=16) plants of Brazilian Green Dwarf coconut. % indicate the percentage of reduction (-) or increase (+) of WS as compared to WW. The values are means (s.e.) of 16 to 24 replicates including the two ecotypes. For each parameter, the letters indicate significant ($P \leq 0.05$) difference by the *F* test.

	LA (m^2)	SLA ($m^2 kg^{-1}$)	TDM (g)	R/S
WW	0.87 (0.02) a	9.79 (0.03) a	660.0 (22.9) a	0.51 (0.01) a
WS	0.64 (0.02) b	9.90 (0.03) a	316.8 (10.5) b	0.39 (0.01) b
%	(-27)	(+1)	(-52)	(-25)

4. Discussion

Photosynthesis (*A*), stomatal conductance (*gs*) and transpiration (*E*) were negligible when the pre-dawn leaf water potential (Ψ_{PD}) reached about -1.2 MPa. *A* and *gs* approached zero when Ψ_{PD} was -3.4 MPa in *Copaifera langsdorffii* (Prado et al., 1994), -2.4 MPa in *Dalbergia miscolobium* Benth. (Sassaki et al., 1997). In some cultivated palm species, photosynthesis approached zero at lower water potential such as -1.9 MPa in peach palm (*Bactris gasipaes* Kunth.) (Oliveira et al., 2002), -2.1 MPa in buriti plants (*Mauritia vinifera* Mart.) (Calbo and Moraes, 1997) and -2.5 MPa in açaí plants (*Euterpe oleracea* Mart.) (Calbo and Moraes, 2000). In other genotypes of coconut this value was found to be -1.35 MPa, on average (Repellin et al., 1997). Although it has not been defined in none of the previous works if the water potential was measured in the pre-dawn, their values were lower than that measured in the current work for green dwarf coconut, suggesting higher sensitivity of the gas exchange variables to soil drought. On the other hand, the results of the present work are in concert with that of other palm species where reduction of *gs* and *E* were observed before significant decreases in *A* (Calbo and Moraes, 1997; Oliveira et al., 2002).

The curvilinear relationship between *A* and *gs* indicate a stomatal limitation of *A* bellow a *gs* of 0.2 mol m^{-2} s^{-1}. Above this value, *A* leveled off and the intrinsic water use efficiency (*A*/*gs*) practically doubled. Stomatal regulation of photosynthesis during mild drought conditions has been well documented (Cornic 2000; Chaves and Oliveira, 2004). In the current study, *Ci* was reduced when *gs* was lower than 0.2 mol m^{-2} s^{-1}, as compared to 0.1 mol m^{-2} s^{-1} in tall coconut genotypes during the dry season, (Prado et al., 2001). While, on one hand, these results confirm the well documented superiority of tall varieties under stress, on the other hand they suggest that is possible to manipulate the stomatal conductance of coconut without great reductions of the carbon assimilation rate, but with expressive improvements of the intrinsic water use efficiency as was recently demonstrated in soybean (*Glycine max* L. Merr.) (Liu et al., 2005). Theoretically, manipulation of water deficit responses, through techniques such as ‘partial rootzone drying’ (Centrito et al., 2005) and ‘regulated deficit irrigation’ (Souza et al., 2005) allows the exploration of the plant’s long distance signaling system, preventing the development of severe water deficit (Davies et al., 2002).

gs = 0.04 mol m^{-2} s^{-1} and Ψ_{PD} = -1.0 MPa, obtained from plots of *A*/*gs* and *Ci* against *gs* and of *A*/*gs* against Ψ_{PD} were the threshold values bellow which *Ci* increased and *A*/*gs* decreased sharply, suggesting the occurrence of non-stomatal limitations under severe water deficit. An increase in *Ci* at low values of gs was observed in peach palm (gs = 0.05 mol m^{-2} s^{-1}, Oliveira et al., 2002) and in other genotypes of coconut (gs = 0.09 mol m^{-2} s^{-1}, Repellin et al., 1997). The low threshold value of *gs* in green dwarf coconut (present results) as compared to that measured by Repellin et al. (1997), may suggest that non-stomatal factors are expected to limit *A* at lower *gs*. However, it must be noted that this lower *gs* was demonstrated to be reached at higher (less negative) Ψ_{PD}. Nevertheless, the non-stomatal factors were demonstrated to limit *A* even after four days of rewatering (see chapter 2). Slow and incomplete recovery of *A* and *gs* upon rewatering were observed in the present work in green dwarf coconut. Similar results were observed in other palms such as buriti (*Mauritia vinifera* Mart.) (Calbo and Moraes, 1997) and açaí plants (*Euterpe oleracea* Mart.) (Calbo and Moraes, 2000). Moreover, the recovery of *gs* was slow when compared to *A*, *E* and Ψ in accordance with what has been observed in other palm species (Calbo and Moraes, 2000; Oliveira et al., 2002). Slow recovery of *gs* after a drought stress period has been attributed to the high concentration of abscisic acid (Mansfield and Davies, 1985), as was demonstrated in the present work.

A high scatter of values of *gs* observed at high Ψ_{PD} suggests closure of stomata before changes in the leaf water status, a response frequently reported in many other plant species (Schulze, 1986; Sassaki et al., 1997; Repellin et al., 1997; Davies et al., 2002; Liu et al., 2005) and also in coconut (Repellin et al., 1997). It was demonstrated, for the first time in drought-stressed coconut, that leaflet abscisic acid concentration ([ABA]) increased before any expressive reduction in Ψ_{PD}. Moreover, a clear relationship between leaf ABA concentration and *gs* was observed at early moments after the imposition of treatments, in spite of a slight delay in the increase of [ABA] after the onset of drought. The delayed increase in [ABA] can be partially explained by observations that xylem rather than leaf ABA concentration is much more sensible to soil drought, as well as, *gs* is thought to respond more to xylem ABA concentration (not measured) than to its accumulation in leaves (Burschka et al., 1983). In addition, it must be noted that is the apoplastic ABA rather than total tissue ABA that regulates stomatal conductance (Schulze, 1986).

Under severe water stress, however, [ABA] increased sharply, probably as a result of the stimulation of ABA biosynthesis in leaves (Pierce and Raschke, 1981). Altered stomatal behavior in coconut plants suffering from leaf scorch decline was recently attributed to increased ABA concentration in the xylem (Ranasinghe, 2005). In leaves of lethal yellowing-affected coconut [ABA] increased 2.5 times when water potential was reduced to -1.8 MPa (León et al., 1996). In this case, [ABA] in symptomless plants was about 3.8 nmol g^{-1} DW, a value slight higher than that measured in the present experiment in well watered plants. It must be noted that León et al. (1996) used adult plants of the tall variety. The variation of [ABA] in WW control plants during the sampling period may be explained by the fluctuating environmental conditions inside the greenhouse, since no control of temperature and irradiance was employed.

During repeated episodes of low turgor, ABA accumulates in the chloroplasts being available for release to the apoplast in response to stress even at levels that may not be severe enough to induce ABA synthesis (Schulze, 1986). Moreover, guard cells cease to be affected by ABA after a period of time (Raschke and Hedrich, 1985). The values of [ABA] in the present experiment were progressively lower from the first to the third cycles. Our results confirm the suspicious of Repellin et al. (1997) that coconut is able to sense the soil water depletion in rootzone and to send a stomata closing signal to leaves. These findings open a new avenue for the research in drought tolerance of coconut: chemical signaling.

In addition to the well established role of ABA in regulation of stomatal conductance, there is evidence that ABA is involved in regulating solute accumulation and thus, osmotic adjustment (LaRosa et al., 1987; Trewavas and Jones, 1991). In the present study, despite its negligible contribution to Ψ_S, proline accumulation in the two ecotypes paralleled the increased in ABA.

The values of osmotic potential measured in WW plants of green dwarf coconut in the current study agree with values measured in other cultivated palm species such as açaí (Calbo and Moraes, 2000) and buriti (Calbo and Moraes, 1997). The net increase in solute concentration, known as osmotic adjustment, allows, by promoting lowering the cell osmotic potential, the maintenance of leaf turgor under water-limited conditions (Morgan, 1984). The osmotic adjustment calculated in the present work for green dwarf coconut (0.05 – 0.24 MPa) was lower than that reported for other species, such as *Stryphnodendron adstringens* (Mart.) Coville, 0.35 MPa (Rocha and Moraes, 1997), açaí, 0.36 MPa (Calbo and Moraes, 2000), wheat (*Triticum aestivum* L.), 0.2-1.4MPa (Morgan, 1984) and rice (*Oryza sativa* L.), 0.4-1.5MPa (Lilley and Ludlow, 1996). However the values found for green dwarf coconut fall within the range obtained for cassava (*Manihot esculenta* Crantz), 0.06-0.44MPa (Alves and Setter, 2004).

Although osmotic adjustment was shown to occur in green dwarf coconut, it did not seem to be important for the turgor maintenance, at least under the severe water stress conditions imposed. In fact, leaf emission and expansion were severely affected by water deficit, as demonstrated by the expressive reduction in the number of leaves in the crown and leaf area, respectively. It is important to point out that osmotic adjustment is always based on an active accumulation of organic solutes at a high cost of energy (Torrecillas et al., 1995). This expenditure of energy could also have affected the growth rate in coconut as pointed out by Sánchez-Blanco et al. (2002).

Proline accumulation in leaves of drought stressed plants has been abundantly documented (Pagter et al., 2005; Türkan et al., 2005). In the current study, proline concentration approximately doubled in relation to control levels, as was observed in other drought-stressed plants such as *Phragmites australis* (Pagter et al., 2005) and species of *Phaseolus* (Türkan et al., 2005). Proline accumulation due to water stress results from a stimulated synthesis, inhibited degradation or an impaired incorporation of proline into proteins (Heuer, 1999). Although the stimulation of proline levels in leaves of drought-stressed coconut was considerable, their contribution to the overall osmotic adjustment was limited, as indicated by two main results: (1) proline concentration was reduced to control levels upon rewatering, whereas osmotic potential did not increase or even decreased in recovered plants and (2) the relative behavior of the two coconut ecotypes for proline accumulation did not reflect their relative behavior in terms of osmotic adjustment. Similar finds were reported recently in callus of durum wheat (*Triticum durum*) cultivars (Lutts et al., 2004) and in water-stressed *Phragmites australis* (Pagter et al., 2005).

Nevertheless, proline is primarily accumulating in the cytoplasm (Aubert et al., 1999), which makes up about 10-20% or less of mature plant cells, so that its importance to osmotic regulation might be higher than that presented here. It has been demonstrated that proline play a more complex role in conferring drought tolerance than in acting as a simple osmolyte. It may protect proteins structure by maintaining their structural stability (Rajendrakumar et al., 1994), act as a free radical scavenger (Reddy et al., 2004), as well as, be involved in the recycling of NADPH via its synthesis from glutamate (Hare and Cress, 1997). By restoring the pool of the terminal electron acceptor of the photosynthetic electron transport chain, proline synthesis may provide some protection against photoinhibition under adverse conditions. High membrane stability in cells of drought-stressed coconut, as indicated by electrolyte leakage measurements (data not shown), as well as, the low drought-induced photoinhibition (see chapter 2) can be explained, at least partially, by the protective role played by proline.

The three consecutive cycles of drying/recovery led to severe reduction of plant growth and dry matter production. Girth size measured at collar and number of leaves emitted are good indicators of the tree's growth and vegetative development, being linked with production, under unchanged environmental conditions (Rognon and Boutin, 1988). In the current work, those two parameters decreased markedly due to drought stress. Reduced dry mass in stressed plants at the end of the experiment, which could be attributed to decreases in both the leaf area and *A*, was observed. As expected in drought stressed plants, the growth rate was affected at the early stages of drought. Interesting, there was not a change in the priority of assimilate allocation in favor to the root system growth (Schulze, 1986). On contrary, in the present study, root growth was more affected by drought treatments (60% of reduction) than shoot growth (48% of reduction), which led to a significant reduction of the root/shoot ratio. This result suggests that increasing the surface for water uptake is not a preferential strategy for green dwarf coconut, although this trait would be interesting for cultivated plants only when the soil water reserve is not limiting (Tardieu, 2005).

5. Conclusions

Our results showed that the imposition of severe water deficit resulted in the activation of physiological responses that culminated in some impairment of carbon assimilation and growth rates. The carbon assimilation rate approached zero at pre-dawn leaf water potential of -1.2 MPa. At mild soil water deficit, *gs* was controlled by ABA accumulated in leaflets and, at severe water deficit, by the leaf water status. Furthermore, water use efficiency was improved at mild water deficit without significant impairment of the photosynthetic rate, suggesting that is possible to manipulate the stomatal conductance within the range of 0.5 to 0.2 mol m^{-2} s^{-1} to bring about an improvement in water economy of the plant. Green dwarf coconut palm showed osmotic adjustment in response to water deficit, which did not avoid dehydration, as well as impairment of growth, one of the most important turgor-driven processes. Expressive accumulation of proline in leaflets was observed in the two ecotypes, which was seemingly related to protective rather than to osmoregulatory functions. Slight differences among the ecotypes with respect to the response to treatments were observed, the JGD showing higher water use efficiency after repeated cycles of water stress, as well as, higher proline accumulation capacity.

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CAPÍTULO 2

Stomatal and non-stomatal limitations to photosynthesis in Brazilian green dwarf coconut recovering from drought stress

Abstract Two greenhouse-grown Brazilian Green Dwarf coconut ecotypes, from contrasting regions in relation to climate, (UGD, from Una, Bahia State and JGD, from Jiqui, Rio Grande do Norte State) were submitted to three consecutive drying/recovery cycles with the objective of discriminating stomatal and non-stomatal effects at the rewatering phase. Upon rewatering for four days, pre-dawn leaf water potential of stressed plants increased from -1.20 to -0.15 MPa on average and only small differences in the internal to atmospheric CO_2 concentration were detected but the photosynthesis rate (A) was not completely recovered to the control values. Light and CO_2-saturated A, carboxylation efficiency, maximum quantum yield of photosystem II and light-saturated electron transport rate were significantly lower in stressed plants. Relative stomatal limitation ranged from 16 to 20% in irrigated plants without significant differences between treatments and between ecotypes. The relative mesophyll limitation was significantly higher in JGD in the cycle 1 but decreased to values significantly lower than in UGD in the other two cycles. The results showed that, after 4 days of rewatering, stomatal limitations of stressed plants were comparable to that of control (~20%), being the incomplete recovery of A after drought stress due to non-stomatal factors. Important differences between the ecotypes were identified, the ecotype JGD showing smaller relative mesophyll limitation, higher apparent quantum efficiency, adjustment in the carbon balance components and signs of acclimation of the photosynthetic capacity after repeated drying/recovery cycles.

Keywords: Chlorophyll *a* Fluorescence; CO_2 response curve; Electron transport rate; Light response curve; Mesophyll limitation; Stomatal conductance

Resumo Dois ecotipos de coqueiro Anão-Verde do Brasil, procedentes de regiões climaticamente contrastantes (Anão-Verde de Una, Bahia, UGD; e Anão-Verde de Jiqui, Rio Grande do Norte, JGD) foram cultivados sob condições de casa de vegetação e submetidos a três ciclos consecutivos de deficiência hídrica com objetivo de discriminar as limitações estomáticas e não-estomáticas à fotossíntese durante a fase de recuperação. Após quatro dias de reidratação, o potencial hídrico foliar medido antes do amanhecer (Ψ_{PD}) aumentou de -1,20 para -0,15 MPa em média. Entretanto, apesar de terem sido detectadas apenas pequenas diferenças na razão entre as concentrações intercelular e atmosférica de CO_2 (Ci/Ca), a taxa fotossintética líquida (A) não recuperou completamente a valores equivalentes aos das plantas controle. Nesse momento, as taxas de fotossíntese saturadas por CO_2 (A_{maxCO2}) e pela radiação fotossinteticamente ativa (*PPFD*) (A_{maxPAR}), a eficiência de carboxilação, a eficiência quântica máxima do PSII e a taxa máxima de transporte de elétrons foram significativamente menores nas plantas estressadas. As limitações estomáticas variaram de 16 a 20% nas plantas controle, sem diferenças significativas entre os tratamentos e entre os ecotipos. As limitações mesofílicas foram significativamente maiores no JGD no ciclo 1 mas decresceram a valores significativamente menores do que no UGD nos dois outros ciclos. Os resultados mostraram que, após quatro dias de reidratação, as limitações estomáticas das plantas estressadas foram comparáveis às das plantas controle (~20%), sendo a recuperação incompleta de A devida a fatores não-estomáticos. Diferenças importantes entre os ecotipos foram identificadas, o JGD mostrando menor limitação mesofílica, maior eficiência quântica aparente e sinais de aclimatação da capacidade fotossintética depois de repetidos ciclos.

1. Introduction

The effects of drought stress on photosynthesis have been thoroughly demonstrated (Lawlor and Cornic, 2002; Tezara et al., 2002; Colom and Vazzana, 2003). There is also consistent information about the effects of water deficit on the relative stomatal and non-stomatal limitations to photosynthesis during the imposition of stress (Escalona et al., 1999; Flexas and Medrano, 2002; Flexas et al., 2002; Lawlor, 2002; Grassi and Magnani, 2005). However, most of these works have shown, as the main result, the responses from an imposed stress, such depletion of soil moisture. Despite the existence of reports on the recovery of the photosynthetic rate from drought stress and the fact that most of then have shown incomplete recovery of photosynthesis immediately after rewatering (Calbo and Moraes, 2000; Oliveira et al., 2002; Souza et al., 2004; Miyashita et al., 2005; Sarker et al., 2005), the contribution of stomatal and non-stomatal factors to the incomplete recovery upon rewatering has not been fully clarified and have been subjected to long-standing debate (Lawlor and Cornic, 2002).

Stomatal closure is often considered as an early physiological response to water deficit which results in decreased net photosynthesis (*A*), through limited CO_2 availability in the mesophyll (Cornic, 2000). However, there is strong evidence that, as water stress increases, photosynthetic processes in the mesophyll such ribulose 1,5-bisphosphate (RuBP) carboxilase/oxygenase (Rubisco) activity, RuBP regeneration, ATP supply, electron transport rate (*J*) and the efficiency of light capture in the photosystems are impaired (Flexas and Medrano, 2002; Lawlor, 2002; Lawlor and Cornic, 2002; Grassi and Magnani, 2005).

The cultivation of dwarf varieties of coconut (*Cocos nucifera* L. '*nana*') has emerged as an important activity in Brazil, which has increased the demand of information about growth, development and yield under different ecological conditions. Being a perennial tree with long productive life span, coconut is exposed to frequent soil and atmospheric drought (Rajagopal and Kasturi Bai, 2002; Gomes et al., 2002). Two Green Dwarf coconut ecotypes from contrasting areas in relation to climate, mainly from Una, Bahia State (UGD) and from Jiqui, Rio Grande do Norte State (JGD), have been largely used for the planting of new areas. The searching for useful characteristics, such as drought recovery ability, to be explored in breeding programs for drought tolerance, as well as, for coconut production in drought-prone areas is of primordial importance. Low stomatal conductance to water vapour (*gs*) and leaf water potential (Ψ_W) with negative consequences for *A* and transpiration (E), as short term responses of coconut to water stress have been demonstrated (Repellin et al., 1994; 1997; Rajagopal and Kasturi Bai, 2002; Passos et al., 1999) but the contribution of non-stomatal factors to the impairment of *A*, mainly at the drought recovery period has not been investigated.

Actually, growing plants with a constant level of water stress is an artificial situation because in nature the soil water content continually changes, as can be observed in humid climates, where the soil water content and the water potential decreases steadily after a rain until the soil is rewetted by another rain (Kramer and Boyer, 1995). Thus, the objective of the present study was to determine the relative contributions of stomatal and non-stomatal factors for the incomplete recovery of photosynthesis in two Brazilian Green Dwarf coconut ecotypes during the recovery phases of simulated drying/recovery cycles in the soil.

2. Material and methods

2.1. Plant material and experimental conditions

Two ecotypes of the Brazilian Green Dwarf cultivar were used in the experiments: Brazilian Green Dwarf from Una (UGD) is cultivated in the southeast region of Bahia State (15º15'S, 39º05'W, 105 masl), which is characterized by hot and humid climate, high indexes of rain fall, air relative humidity above 80% and mean temperature between 24 and 27ºC (Almeida e Santana, 1999); Brazilian Green Dwarf from Jiqui (JGD) is cultivated in the Rio Grande do Norte State (05º47'S, 35º12'W, 30 masl), a contrasting semi-arid environment characterized by high temperatures (maximum of 33ºC and minimum of 23ºC), low pluviometric precipitation (< 650 mm in one year) and average air relative humidity of 69% (Carmo Filho et al., 1991). The plants were cultivated in pots of 150 L filled with a soil/sand mixture (1:2), under greenhouse conditions at the Unidade de Crescimento de Plantas of the Federal University of Viçosa, Brazil (20º 45’S, 42º 52’W, 648m asl). During the experiment, the daily average air temperature, measured with a non-linear thermistor connected to a data logger LI-1200 Data Set Recorder (LI-COR, Inc. USA), was 23.1^{o}C (maximum of 30.5ºC and minimum of 18.6ºC). Relative humidity, measured with a "harp" of human hair sensor type of a thermo-hygrograph calibrated weekly against a ventilated wet bulb psycrometer, was 74.6% on average. The plants were irrigated daily to maintain a soil water content close to field capacity (-0.002 MPa). The soil water potential was estimated after the construction of a soil water retention curve, from which the field capacity was estimated following the moisture equivalent method (Ruiz et al., 2003). All essential nutrients were supplied, following the leaf and soil analyzes and the requirements of the specie (Magat, 2003). When the plants were one year old the drought treatment was initiated by suspension of irrigation until the net photosynthetic rate (*A*) approached zero (soil water potential -0.52 MPa), which was considered the point of maximum stress. The plants were then rewatered for eighth days until the assimilation rates were > 85% of the control. This procedure was repeated two times, giving a total of 3 successive drying/recovery cycles. The drought period lasted 44, 35 and 35 days in the cycles 1, 2 and 3, respectively. For each ecotype, 20 plants (12 stressed and 8 controls) were organized in a completely randomized design. The term cycle (1, 2 and 3) was used in the present work for indicating the 4th day after rewatering.

2.2. Gas exchange measurements

The measurements were done from 8:30 to 11:30 h a.m. using a Portable Photosynthesis System LI-6400 (LI-COR Biosciences Inc., Nebraska, USA) equipped with an artificial irradiance source 6400-02B RedBlue. Light response curves were carried out with eight levels of *PPFD* (0, 50, 100, 200, 400, 800, 1200 and 1600 µmol photon m^{-2} s^{-1}) in a decreasing order, with 2-3 min intervals between each reading. The CO_2 flux was adjusted to maintain an inside chamber concentration of 380 µmol mol^{-1}. The CO_2 response curves were measured under a *PPFD* of 1200 µmol photon m^{-2} s^{-1}, since it was preliminarily proved to be above the photosynthesis light saturation intensity for all measured leaves. Different CO_2 concentrations were obtained from the 12 g CO_2 cartridges and automatically controlled by the CO_2 injector device of LI-COR 6400 (6400-01 CO_2 injector, LI-COR, USA). The sequence of measurements initiated at an in chamber CO_2 concentration of 50 µmol mol^{-1}, increasing to 100, 200, 380, 600, 800 and 1000 µmol mol^{-1} at 3-4 min intervals (Escalona et al., 1999). These rapid A/Ci curves were chosen since the objective was to measure CO_2-driven changes in A and not in gs. The values of A were stable (CV < 1%) in 3-4 min after each change of the in chamber CO_2 concentration. All measurements were done in attached leaflets at the middle of the rank two leaf (counting from the top, taking the spindle as zero) of three or five different plants for each treatment. The intercellular CO_2 concentration (Ci) was calculated by the equipment from the values of A, gs and transpiration (E) (von Caemmerer and Farquhar, 1981). The relation between intercellular to atmospheric CO_2 concentration (Ci/Ca) was calculated from the values of Ci and Ca of the A *vs.* *PPFD* curves when *PPFD* > 800 µmol photons m^{-2} s^{-1}. The leaf chamber (6 cm^2) temperature was maintained at 26ºC and the leaf-to-air water vapour pressure deficit (*VPD*) was 1.0 ± 0.2 kPa, on average, during the measurements. The relative stomatal limitation (L_S) was calculated from A *vs.* Ci curves, as $L_S = 100 \times (A_0 - A)/A_0$, where A and A_0 are the photosynthetic rate at ambient Ca (380 µmol mol^{-1}) and at $Ci = Ca$, respectively (Farquhar and Sharkey, 1982). The relative mesophyll limitation (Lm) was calculated as $Lm = 100 \text{ x } (A\text{c} - A_{\text{S}})/A\text{c}$, where Ac and A_{S} are CO_2-saturated A (Ci = 800 µmol mol^{-1}) of control and stressed leaves, respectively (Jacob and Lawlor, 1991).

2.3. Chlorophyll a fluorescence measurements

Instant light response curves of fluorescence were measured on the same attached leaflets (n = 3 - 4) used for gas exchange measurements, using the light-curve program of the Mini PAM fluorometer (Walz, Effeltrich, Germany) (Rascher et al., 2000). The leaflets where dark adapted for 30 min before the light curve runs, when the actinic light intensity was increased from 0 to 1600 µmol m^{-2} s^{-1} in nine steps of 30 s. Apparent rate of electron transport of PSII (J) was estimated as $J = \Phi_{PSII} \times PPFD \times a \times 0.5$, where Φ_{PSII} is the effective quantum yield of PSII, a is the fraction of incident *PPFD* absorbed by the leaflet, assumed to be 0.84 (Ehleringer, 1981) and the factor 0.5 assumes equal excitation of both PSI and PSII (Laisk and Loreto, 1996). Φ_{PSII} was calculated as (Fm' – F)/Fm', where F is fluorescence yield of the light-adapted sample and Fm' is the maximum light-adapted fluorescence yield when a saturating light pulse of 800 ms of duration (intensity > 4000 µmol m^{-2} s^{-1}) is superimposed on the prevailing environmental light levels. The maximum quantum yield of PSII, [Fv/Fm = (Fm – F_0)/Fm)] was calculated using the so called dark-adapted parameters F_0 and Fm (obtained after the first saturating pulse of the light curves) where F_0 is the basal fluorescence yield measured after the illumination of a dark adapted sample with a measuring light (< 0.1 µmol m^{-2} s^{-1}, > 650 nm) and Fm is the maximum fluorescence yield of a dark adapted sample obtained following a saturating light pulse (Rohácek, 2002).

2.4. Leaflet water status and pigments content

At the point of maximum stress and after rewatering for four days, Ψ_{PD} was measured at 4:30 a.m. in one leaflet per plant of four plants per treatment, with a pressure chamber Skye SKPM 1400 (Skye Instruments LTD., UK). All measurements were done on opposite leaflets of those used for gas exchange measurements, following the methodology described by Milburn and Zimmermann (1977). Briefly, after detaching the leaflet, the base of the lamina was trimmed with a sharp knife to free the cylindrical midrib, the leaflet was rolled (keeping the adaxial surface outermost to avoid breaking the rib) and sealed into the pressure chamber. The pressure was increased slowly (0.02 MPa s^{-1}) with compressed nitrogen until the sap was visible at the exposed base of the midrib.

Concentration of pigments per area was determined in dimetyl sulphoxide (DMSO) extracts of leaflet discs after Hiscox and Israelstam (1979), with some modifications. After incubation of 3 leaf discs (0.5 cm) with 5 mL of $CaCO_3$-saturated DMSO at 65ºC for 48 h, the absorbance of extracts was read in a Hitachi U-2000 double-beam spectrophotometer (Hitachi Instruments Inc, Danbury, CT), and total chlorophylls (Chl) and carotenoids were calculated using the equations proposed by Wellburn (1994) for DMSO extracts.

2.5. Modeling and statistics

Non-linear regression to exponential models (see chapter 3) were used to estimate the photosynthetic parameters. For *A vs. PPFD* the model used was (Iqbal et al., 1997):

$$A = A_{\mathrm{maxPAR}} \times (1 - \exp(- \alpha \times (PPFD/A_{\mathrm{maxPAR}})) - R_{\mathrm{d}} + \varepsilon$$

where A_{maxPAR} is the light-saturated photosynthetic rate, α is the apparent quantum yield, R_{d} is the dark respiration rate (estimated as the *y*-intercept of a linear regression of the first points of *A vs. PPFD* curves) and ε is the random error. For *A vs. Ci* curves the equation used was (Escalona et al., 1999):

$$A = A_{\mathrm{maxCO2}} \times (1 - \exp(- \mathrm{k} \times (Ci - \Gamma_{\mathrm{CO2}})) + \varepsilon$$

where A_{maxCO2} is the CO_2-saturated photosynthetic rate, k is a constant of proportionality and Γ_{CO2} is the compensation point for CO_2. The equation used for *J vs. PPFD* was the same of *A vs. PPFD*, where *A* and A_{maxPAR} were replaced by *J* and J_{max}, respectively (Rascher et al., 2000). Light respiration rate (R_{L}) and carboxylation efficiency (CE), respectively the *y*-intercept and the slope of *A vs. Ci*, were estimated by a linear regression of the first points of the *A vs. Ci* curves (Escalona et al., 1999). The photosynthetic parameters were compared using a test of identity of models (Cunia et al., 1973), whose procedure was extrapolated for non-linear-models. For each individual replicate, the values of compensation irradiance (Γ_{light}, *x*-intercept), estimated by a linear regression of the first points of the *A vs. PPFD* (Escalona et al., 1999), Ψ_{PD}, *Lm*, L_S, pigments, F_0 and Fv/Fm were submitted to ANOVA and, when indicated, to the Tukey’s mean comparison test, at 5% of probability. All mathematical procedures were performed using the software Statistica (Statsoft Inc.).

3. Results

3.1 Gas exchange and water relations

The progressive soil water reduction led to decreases in Ψ_{PD} to values of -1.22 ± 0.04 MPa, on average, at the point of maximum stress (Fig. 1). Upon rewatering, the mean value of Ψ_{PD} increased to -0.15 ± 0.01 MPa, at the 4th day of recovery, although the values in stressed plants of JGD still were significantly lower (more negative) in the cycles 1 and 2, when compared to irrigated plants (Fig. 1). There were not significant differences in Ψ_{PD} between ecotypes.

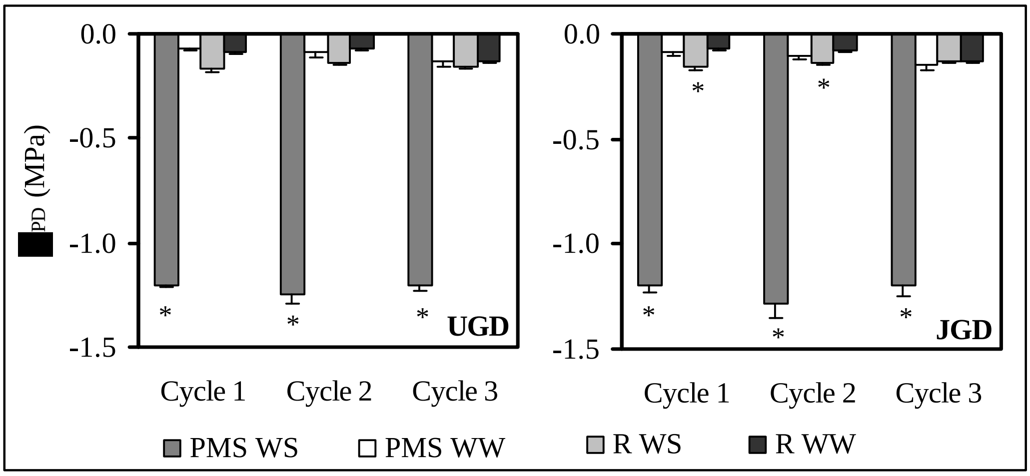

Fig. 1. Pre-dawn leaf water potential measured in well watered (WW) and water stressed (WS) plants of Brazilian Green Dwarf coconut from Una (UGD) and Jiqui (JGD) at the point of maximum stress (PMS) and at the 4th day of recovery (R). Values are means (n = 4-6, s.e.) and * indicate significant ($P \leq 0.05$) difference between WW and WS, by the *F* test.

A and *gs*, measured at ambient CO_2 concentration (Ca = 380 µmol mol^{-1}) and saturating irradiance ($PPFD$ = 1200 µmol m^{-2} s^{-1}) decreased in stressed plants of the two ecotypes, and were not completely recovered four days after rewatering (Table 1). When compared to control, *A* and *gs* of stressed plants were 63 and 42% in UGD in the cycle 1, respectively (Table 1). In JGD, *A* and *gs* of stressed plants were 57 and 32% of their controls, respectively. The two ecotypes, mainly JGD, showed a less pronounced effect of drought on *A* with the imposition of successive cycles, as indicated by the percentage of decrease of stressed in relation of irrigated plants. That difference was even clearer for *gs*. While the percentage of reduction in *gs* was approximately constant in UGD, stressed plants of JGD showed a markedly increase in the *gs* recovery speed after the successive drying/recovery cycles, the differences between control and stressed being non-significant in the cycle 3. Despite the low *gs*, only small reductions of *Ci*/*Ca* (< 8%) were observed, which were significant for UGD in the cycle 3 and for JGD in the cycles 1 and 2 (Table 2).

Table 1. Net photosynthesis (*A*), stomatal conductance to water vapour (*gs*) and intercellular to atmospheric CO_2 concentration (*Ci/Ca*), measured under saturating irradiance (*PPFD* = 1200 µmol photons m^{-2} s^{-1}) and ambient CO_2 concentration (*Ca* = 380 µmol CO_2 mol^{-1} air) in Brazilian Green Dwarf coconut leaflets at the 4th day of recovery from water deficit. The values are mean (s.e.) of 8 – 12 replicates.

	Cycle 1			Cycle 2			Cycle 3		
	WW	WS	%	WW	WS	%	WW	WS	%
A (µmol CO_2 m^{-2} s^{-1})									
UGD	11.8 (0.4) ^{a}	7.4 (0.5) ^{b}	63	14.0 (0.3) ^{a}	11.5 (0.4) ^{b}	82	13.1 (0.1) ^{a}	9.9 (0.4) ^{b}	76
JGD	12.3 (0.5) ^{a}	7.0 (0.4) ^{b}	57	13.8 (0.4) ^{a}	10.9 (0.4) ^{b}	79	12.9 (0.2) ^{a}	11.8 (0.2) ^{b}	91
gs (mol H_2O m^{-2} s^{-1})									
UGD	0.26 (0.02) ^{a}	0.11 (0.01) ^{b}	42	0.34 (0.06) ^{a}	0.20 (0.02) ^{b}	59	0.45 (0.02) ^{a}	0.21 (0.03) ^{b}	47
JGD	0.28 (0.02) ^{a}	0.09 (0.01) ^{b}	32	0.34 (0.02) ^{a}	0.18 (0.02) ^{b}	53	0.36 (0.01) ^{a}	0.29 (0.03) ^{a}	81
Ci /*Ca*									
UGD	0.75 (0.01) ^{a}	0.68 (0.02) ^{a}	91	0.76 (0.03) ^{a}	0.71 (0.01) ^{a}	93	0.82 (0.01) ^{a}	0.74 (0.02) ^{b}	90
JGD	0.76 (0.01) ^{a}	0.63 (0.01) ^{b}	83	0.77 (0.01) ^{a}	0.68 (0.02) ^{b}	88	0.78 (0.02) ^{a}	0.77 (0.02) ^{a}	99

UGD, ecotype from Una; JGD, ecotype from Jiqui; WW, well watered; WS, water stressed; %, percentage in relation to control. For each variable and ecotype, different letters indicate significant ($P \leq 0.05$) differences between WW and WS, by the *F* test

3.2. PPFD and CO_2 response curve parameters

The values of A_{maxPAR} were significantly lower in stressed plants after four days of rewatering, reaching 73 and 83% of their controls in the cycle 1 and 86 and 89% in the cycle 3, in UGD and JGD, respectively (Table 2). The apparent quantum yield (α) was significantly reduced in stressed plants of UGD in the three cycles, but the drought effect became less accentuated with the imposition of the other cycles (Table 2). Only small non-significant reductions of α were observed in JGD. A markedly decrease of R_d with the imposition of repeated cycles was observed, which was more pronounced in JGD (from 94 to 46% in relation to their controls) (Table 2). However, the reductions were not significant. Γ_{PAR} was not affected by the drying/recovery treatments, although a decreasing trend was observed, which was, again, more pronounced in JGD than in UGD (Table 2).

Table 2. Light response curves parameters estimated for Brazilian Green Dwarf coconut at the 4th day of recovery from water deficit. The values are mean (s.e.) of 3 – 5 replicates

	Cycle 1			Cycle 2			Cycle 3		
	WW	WS	%	WW	WS	%	WW	WS	%
A_{maxPAR} (µmol CO_2 m^{-2} s^{-1})									
UGD	14.9 (0.2) ^{aA}	10.9 (0.3) ^{bA}	73	15.2 (0.2) ^{aA}	12.7 (0.2) ^{bA}	84	15.2 (0.2) ^{aA}	13.0 (0.2) ^{bA}	86
JGD	13.8 (0.2) ^{aB}	11.4 (0.2) ^{bA}	83	14.2 (0.2) ^{aB}	11.4 (0.2) ^{bB}	80	14.3 (0.2) ^{aB}	12.7 (0.2) ^{bA}	89
α (µmol CO_2 $mmol^{-1}$ photons)									
UGD	54.6 (2.0) ^{aA}	35.6 (1.8) ^{bA}	65	54.2 (1.9) ^{aA}	42.9 (1.7) ^{bA}	79	55.0 (2.0) ^{aA}	45.4 (1.9) ^{bA}	83
JGD	54.5 (1.8) ^{aA}	49.9 (1.9) ^{aB}	92	47.8 (2.0) ^{aB}	42.1 (2.1) ^{aA}	88	53.9 (1.9) ^{aA}	50.6 (2.0) ^{aA}	94
R_d (µmol CO_2 m^{-2} s^{-1})									
UGD	0.77 (0.14) ^{aA}	0.72 (0.14) ^{aA}	93	0.80 (0.16) ^{aA}	0.57 (0.14) ^{aA}	71	0.60 (0.15) ^{aA}	0.46 (0.15) ^{aA}	77
JGD	0.64 (0.18) ^{aA}	0.60 (0.15) ^{aA}	94	0.62 (0.27) ^{aA}	0.34 (0.23) ^{aA}	55	0.74 (0.18) ^{aA}	0.34 (0.21) ^{aA}	46
Γ_{PAR} (µmol photons m^{-2} s^{-1})									
UGD	16.1 (1.7) ^{aA}	21.7 (3.2) ^{aA}	135	16.7 (1.4) ^{aA}	15.1 (2.1) ^{aA}	91	13.4 (3.0) ^{aA}	10.7 (2.2) ^{aA}	80
JGD	13.9 (0.2) ^{aA}	13.8 (1.3) ^{aA}	99	15.1 (1.8) ^{aA}	11.5 (5.0) ^{aA}	76	15.4 (2.5) ^{aA}	10.5 (0.9) ^{aA}	68

A_{maxPAR}, light-saturated photosynthetic rate; α, apparent quantum yield; R_d, dark respiration rate; Γ_{PAR}, compensation irradiance; UGD, ecotype from Una; JGD, ecotype from Jiqui; WW, well watered; WS, water stressed; %, percentage in relation to control. For each parameter, lower case letters indicate significant differences between WW and WS within each ecotype and capital letters indicate significant differences among ecotypes within each treatment, by the *F* test ($P \leq 0.05$).

The water deficit led to significant reductions in A_{maxCO2} and J_{max} in the two ecotypes (Table 3). With the imposition of the cycles, the values of A_{maxCO2} of stressed plants increased from 65 to 82% of the control in JGD but stayed at 74 - 71% in UGD (Table 3). Carboxylation efficiency (CE) was also incompletely recovered at the moment of measurements, but significant reductions were observed only in the cycle 1, for the two ecotypes, and in the cycle 3 for UGD. A less accentuated decrease with the imposition of cycles was also observed for J_{max} in the two ecotypes, but the values of the stressed plants still were significantly reduced (46% in UGD and 32% in JGD) at the end of the experiment (Table 3). Significant effect of drought on R_L was observed only in UGD in the cycle 1. There were not significant effects of water deficit on Γ_{CO2} for the two ecotypes (Table 3).

Table 3. CO_2 response curves parameters estimated for Brazilian Green Dwarf coconut at the 4th day of recovery from water deficit. The values are mean (s.e.) of 3 – 5 replicates

	Cycle 1			Cycle 2			Cycle 3		
	WW	WS	%	WW	WS	%	WW	WS	%
A_{maxCO2} (µmol CO_2 m^{-2} s^{-1})									
UGD	19.0 (0.5) ^{aB}	14.1 (0.5) ^{bA}	74	23.5 (0.7) ^{aA}	16.7 (0.5) ^{bB}	71	21.8 (0.5) ^{aA}	15.4 (0.3) ^{bB}	71
JGD	24.3 (0.8) ^{aA}	15.7 (0.9) ^{bA}	65	24.6 (0.9) ^{aA}	20.8 (0.7) ^{bA}	85	20.9 (0.5) ^{aA}	17.1 (0.5) ^{bA}	82
ε (mmol m^{-2} s^{-1})									
UGD	53.2 (3.3) ^{aB}	41.1 (2.7) ^{bA}	77	64.1 (4.1) ^{aA}	54.1 (4.1) ^{aA}	84	59.9 (2.0) ^{aA}	52.2 (2.0) ^{bA}	87
JGD	66.5 (3.7) ^{aA}	47.7 (5.0) ^{bA}	72	67.2 (3.4) ^{aA}	65.5 (3.7) ^{aA}	97	65.0 (5.3) ^{aA}	60.0 (5.1) ^{aA}	92
R_L (µmol CO_2 m^{-2} s^{-1})									
UGD	3.65 (0.34) ^{aA}	2.67 (0.30) ^{bA}	73	3.57 (0.42) ^{aA}	3.33 (0.43) ^{aA}	92	3.38 (0.22) ^{aA}	3.01 (0.21) ^{aA}	89
JGD	4.01 (0.39) ^{aA}	2.81 (0.51) ^{aA}	70	3.94 (0.36) ^{aA}	3.97 (0.38) ^{aA}	103	3.66 (0.57) ^{aA}	3.52 (0.53) ^{aA}	96
Γ_{CO2} (µmol CO_2 mol^{-1} air)									
UGD	71.2 (3.5) ^{aA}	68.1 (4.7) ^{aA}	96	58.4 (3.7) ^{aA}	65.3 (3.7) ^{aA}	112	59.8 (3.2) ^{aA}	60.9 (3.0) ^{aA}	102
JGD	62.1 (4.7) ^{aA}	63.2 (7.2) ^{aA}	102	60.5 (3.8) ^{aA}	61.7 (3.9) ^{aA}	102	59.5 (4.1) ^{aA}	61.0 (3.8) ^{aA}	103
J_{max} (µmol electrons m^{-2} s^{-1})									
UGD	94.2 (2.3) ^{aA}	42.2 (1.8) ^{bA}	45	104.1 (2.9) ^{aA}	82.8 (2.4) ^{bB}	80	104.3 (2.6) ^{aA}	56.3 (1.5) ^{bB}	54
JGD	94.8 (3.4) ^{aA}	42.0 (1.9) ^{bA}	44	106.7 (1.6) ^{aA}	87.9 (1.5) ^{bA}	82	110.2 (4.0) ^{aA}	75.2 (2.2) ^{bA}	68

A_{maxCO2}, CO_2-saturated photosynthetic rate; CE, carboxylation efficiency; R_L, light respiration rate; Γ_{CO2}, CO_2 compensation point; J_{max}, light-saturated electron transport rate; UGD, ecotype from Una; JGD, ecotype from Jiqui; WW, well watered; WS, water stressed; %, percentage of reduction in relation to control. For each parameter, lower case letters indicate significant differences between WW and WS within each ecotype and capital letters indicate significant differences among ecotypes within each treatment, by the *F* test ($P \leq 0.05$).

3.3. Stomatal vs. non-stomatal limitations

The relative stomatal limitation to *A* (L_S) ranged from 16 to 19% in irrigated plants of UGD and from 16 to 18% in JGD (Table 4). Non-significant increases of L_S were observed in stressed plants of JGD in all three cycles (Table 4). From cycle 1 to 3, the relative mesophyll limitation (*Lm*) decreased from 32 to 19% in JGD, but did not vary (~24%) in UGD. The ecotype JGD showed a significantly higher *Lm* at the first cycle when compared to UGD, the inverse being observed at the other two cycles (Table 4).

Table 4. Relative stomatal (L_S) and mesophyll (*Lm*) limitations to photosynthesis in Brazilian Green Dwarf coconut recovering from water deficit. The values are mean (s.e.) of 3 – 5 replicates

Cycle	L_S (%)				*Lm* (%)	
	UGD		JGD		UGD	JGD
	WW	WS	WW	WS		
1	16.8 (1.2) ^{aA}	16.2 (2.0) ^{aA}	18.2 (1.1) ^{aA}	22.0 (0.4) ^{aA}	23.5 (1.3) ^{B}	31.9 (0.3) ^{A}
2	19.4 (1.2) ^{aA}	19.2 (0.6) ^{aA}	18.1 (0.9) ^{aA}	19.0 (0.4) ^{aA}	18.1 (0.6) ^{A}	11.0 (1.2) ^{B}
3	15.5 (0.4) ^{aA}	17.4 (1.1) ^{aA}	16.4 (0.5) ^{aA}	19.6 (0.8) ^{aA}	23.4 (0.6) ^{A}	18.7 (0.8) ^{B}

UGD, ecotype from Una; JGD, ecotype from Jiqui; WW, well watered; WS, water stressed; For L_S, lower case letters indicate significant differences between WW and WS within each ecotype and capital letters indicate significant differences among ecotypes within each treatment, by the *F* test ($P \leq 0.05$); For *Lm* different capital letters indicate significant ($P \leq 0.05$) differenced among ecotypes, within each cycle, by the *F* test.

3.4. Pigments, Fv/Fm and F_0

Drought stress led to reductions of Chl contents per unit area, which were significant only for UGD (Fig. 2). Significant reduction of Chl content for JGD was observed only in the cycle 2. Except for UGD in the cycle 3, only small non-significant effects of drought stress on carotenoids were observed (Fig. 2). The average Fv/Fm for all measurements was 0.83 ± 0.01 in control plants. Significant reductions of this parameter were observed in stressed plants of the two ecotypes in the cycles 1 and 2. The values of F_0 showed an increasing trend in stressed plants of the two ecotypes, although significant differences have been observed only in the cycle 1 (Fig. 2).

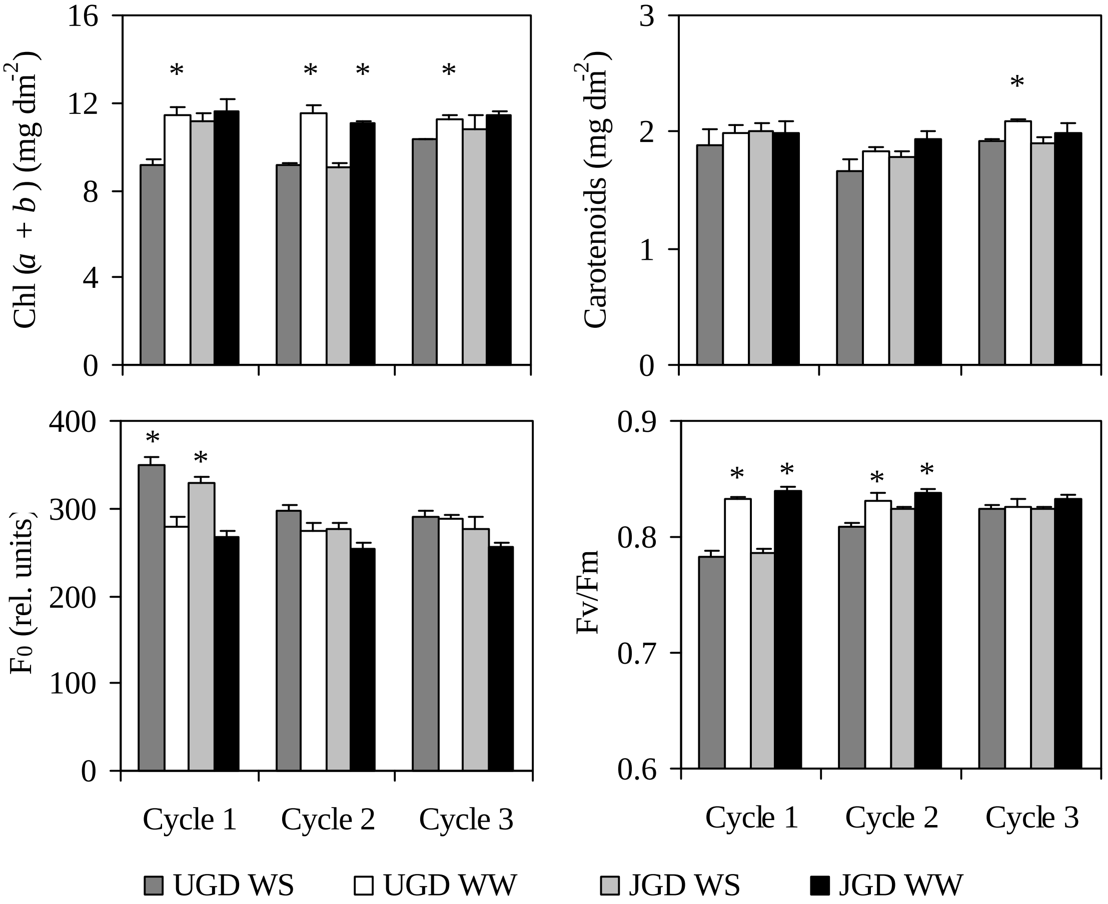

Fig. 2. Total chlorophylls (Chl) and carotenoids concentration and fluorescence parameters measured in well watered (WW) and water stressed (WS) plants of Brazilian Green Dwarf coconut from Una (UGD) and Jiqui (JGD) recovering fron water deficit. Values are means (n = 4-6, s.e.) and * indicate significant ($P \leq 0.05$) difference between WW and WS, within each ecotype, by the *F* test.

4. Discussion

A rapid increase in *A* of water-stressed plants upon rewatering indicates that the basis of both biochemical and photochemical mechanisms is not impaired by drought, leading to the conclusion that the decreases in *A* are only the result of stomatal closure (Cornic, 2000). The present results showed that *A* and *gs* were not fully recovered after four days of rewatering, despite the almost complete recovery of *Ci*/*Ca*. Even though stomatal closure seemed to be the main factor limiting *A* in young coconut subjected to edaphic drought, after prolonged stress the non-stomatal factors seemed to be important in photosynthesis regulation (Reppellin et al., 1994, 1997). It has been reported that α and A_{maxPAR} could be affected by low *gs* (Escalona et al., 1999), but their decreases in stressed plants, as demonstrated here mainly in UGD, might also be resulted from the drought effects on both the light harvesting efficiency and the photosynthetic electron transport system (Tezara et al., 2003). Miyashita et al., (2005) considered that a complex pool of factors including injury to electronic transfer in PSII and damage to the chloroplast and mitochondrial structure, may be involved in the incomplete recovery of photosynthesis in kidney bean under drought stress. Negligible effects of soil water deficit on Γ_{PAR} and R_d, were observed, although their decreasing trend seems to be advantageous because it may help maintain a positive carbon balance under low efficiency of light capture, revealing that, after successive drying/recovery cycles the photosynthetic capacity is down-regulated in dwarf coconut, mainly in JGD.

In *A*/*Ci* curves, which eliminate the effect of decreased *gs* induced by drought, A_{maxCO2} equates to the maximum rate of RuBP regeneration (corresponding to a maximum *J*), as well as, decreased activity and/or amount of Rubisco would be seen as a decrease in CE (Farquhar et al., 1980; von Caemmerer and Farquhar, 1981). Water deficit has been shown to decrease the electron flux (*J*) through PSII in a small proportion of the decrease in *A* (Lawlor and Cornic, 2002). Such effect was observed in the present work only for UGD in the cycle 2, where the decrease of J_{max} (20%) was proportionally less than that of A_{maxCO2}, (29%), suggesting the occurrence of a transient increase of the sink for electrons (probably photorespiration), as was observed in sunflower under water deficit and ambient CO_2 concentration (Tezara et al., 2002). It must be noted that only A_{maxCO2} and not J_{max} was measured under non-photorespiratory conditions.

There are uncertainties in calculating *Ci* in water stressed leaves due to stomatal patchiness and/or to increasing importance of cuticular transpiration as stomata close (Cornic, 2000; Lawlor, 2002). While we cannot be sure that our *Ci* values were not subject to that problems, there are plenty of reports attributing less importance to stomatal patchiness and more to metabolic impairment caused by water stress (Lawlor and Cornic, 2002; Tezara et al., 2003). Despite the fact that heterogeneous stomatal closure has not been studied in coconut, it should be noted that: (1) patchiness should not be an important problem in slowly dehydrating leaves, as was the case of the present study (Lawlor, 2002) and (2) the leaves of most monocotyledonous species are of the homobaric type (Kawamitsu et al., 1993). Patchy CO_2 assimilation patterns may occur in heterobaric leaves, whose extensions of bundle sheath cells to the upper and lower epidermis delimits the mesophyll laterally so that the CO_2 diffusion across these vascular extensions is restricted (Gunasekera and Berkowitz, 1992). In addition, leaves of monocotyledonous contain more stomata per unit of area, which contributes for homogenizing the internal pressure of CO_2 (Kawamitsu et al., 1993). On the other hand, the influence of both patchiness and cuticular transpiration on *Ci* in the present study was assumed to be of minor relevance, since *gs* measured at the fourth day of recovery was higher than 0.03 mol m^{-2} s^{-1}, bellow which that phenomenon are expected to be important (Boyer, et al., 1997; Flexas et al., 2002; Grassi and Magnani 2005).

The photosystem II activity has been shown to be quite resistant to drought in many species (Blaikie and Chacko, 1998; Colon and Vazzana, 2003; Tezara et al., 2003). In the present experiment, severe water stress led to chronic photoinhibition in the two ecotypes of dwarf coconut, as indicated by reductions of Fv/Fm, A_{maxPAR} and α. It has been demonstrated that not only high irradiance *per se*, but also any environmental constraint directly or indirectly limiting the photosynthetic capacity is likely to induce photoinhibition (Pastenes et al., 2004). On the other hand, the reductions in light harvest capacity due to the reduction in pigments contents and the similar values of Fv/Fm at the end of experiment (cycle 3) may suggest that down-regulation of photochemistry rather than damage to PSII is occurring (Giardi et al., 1996; Pastenes et al., 2004). It must be noted that the decreasing values of air temperature and, mainly, solar irradiance towards the end of experiment seems to have contributed for the lack of drought effects on Fv/Fm in the cycle 3. Interactive effects of drought and high-light stresses, as suggested by our results, have been shown to occur in both shade and sun phenotypes of the same plant species, being, therefore, dependent on the species-phenotype combination (Valladares et al., 2004). Nevertheless, the reduction in Fv/Fm may be due, in part, to the increase in F_0 (Fig. 2). Whereas increased F_0 may indicate energy dissipation (fluorescence) in the complex antenna (Strasser et al., 2000), the contribution of photosystem I to the signal of F_0, which could be significant in stressed plants (Pfündel, 1998), cannot be ruled out.

The values of L_S calculated for irrigated plants of Green Dwarf coconut (from 16 to 19%) are lower than that obtained for other non-stressed C_3 species, such *Vitis vinifiera* L (Escalona et al., 1999) and *Helianthun annuus* L. (Tezara et al., 2002) (> 20%) and *Lycium nodosum* Miers. (~ 35%) (Tezara et al., 2003). Low values of L_S suggest that the dwarf coconut has not an efficient stomatal control of water loss, as reported elsewhere (Rajagopal et al., 1989; Reppellin et al., 1994, 1997; Passos et al., 1999). Despite significantly lower than in irrigated plants in all three cycles (Table 2), *gs* of stressed plants had recovered to a value (> 0.09 mol $m^{-2}s^{-1}$) above which only small increases in *Ci* were observed (data not shown), which explain the minor differences observed between treatments for *Ci*/*Ca* and L_S. Rather than negligible stomatal limitation, this mean that the resistance to CO_2 influx into the leaves of the recovering plants was proportionally the same as that of control plants, the large differences in *A* between treatments being attributed to non-stomatal factors. Indeed, the range of *Lm* values presented here for Green Dwarf coconut (11-32%) agree with the values calculated for sunflower under mild water stress (15-18%) (Tezara et al., 2002) and for *Lycium nodosum* Miers. after 5 days without irrigation (18-32%) (Tezara et al., 2003). Reductions in the differences of J_{max}, A_{maxCO2} and CE between stressed and control plants with the imposition of successive drying/recovery cycles can explain the decrease of *Lm* in JGD, possibly through an adjustment of the photosynthetic machinery (Flexas and Medrano, 2002; Lawlor and Cornic, 2002). It must be noted that *Lm*, in the form that was calculated, is a measure of the capacity of mesophyll to fix CO_2 at saturating *Ci*, its value being zero in control plants (Tezara et al., 2003).

The method used in the present work for calculating L_S and *Lm* (mainly based on the elimination approach, Jones, 1985) do not allow separating the diffusive (mesophyll conductance) and the truly biochemical components of the non-stomatal limitations (Grassi and Magnani, 2005). However, whereas our results are in concert with the reports of other authors, where non-stomatal limitations increased in response of water stress (Escalona et al., 1999; Tezara et al., 2002; Tezara et al., 2003; Souza et al., 2004), they showed that non-stomatal factors were important even after 4 days of rewatering. That result, associated to the photochemical impairment, contributed to incomplete recovery of *A*. Although only the decreases in A_{maxCO2}, CE and J_{max} may be insufficient to indicate metabolic impairment of *A* (Centritto, 2005), the present results suggest that the regeneration of RuBP (as indicated by reduced A_{maxCO2}) as well as, the Rubisco amount and/or activity (as indicated by reduced CE) (Farquhar et al., 1980) seem to have been impaired by the drought treatment. The limitation of RuBP regeneration has been considered as the first response of the mesophyll to water stress (Escalona et al., 1999). While the present experiment offers a first approach toward the estimation of non-stomatal limitation to photosynthesis in coconut recovering from drought stress, specific experiments that can discriminate the diffusive and the biochemical components are needed to strengthen and to validate our results.

In conclusion, it was demonstrated that, in the two dwarf coconut ecotypes studied, *A* did not recover to control levels after 4 days of rewatering despite the low differences between treatments for *Ci*/*Ca*. Whereas stomatal closure contributed to limiting photosynthesis even in irrigated palms, non-stomatal limitations were shown to be important, indicating that photosynthesis is co-limited by stomatal and non-stomatal factors. Reduced CO_2-saturated *A*, carboxylation efficiency and electron transport rate configure an initial picture of the non-stomatal limitation to photosynthesis in coconut. In addition, photoinhibition was shown to be an important component of the pool of limitations to *A* in dwarf coconut under drought stress. Important differences between the ecotypes were identified, the ecotype JGD showing smaller relative mesophyll limitation, faster recovery of apparent quantum efficiency, adjustment of the carbon balance components (reduction in dark respiration and light compensation point) and signs of acclimation of the photosynthetic capacity after repeated drying/recovery cycles.

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CAPÍTULO 3

Photosynthetic irradiance-response in leaves of dwarf coconut palm (*cocos nucifera* l. ‘*nana*’, Arecaceae): comparison of three models

Abstract Experimental data of irradiance-response curves of photosynthesis, obtained from independent experiments with the Brazilian Green Dwarf coconut palm, ecotype from Una, Bahia (UGD), were used to fit three empirical models with the aim of comparing their adequacies. The non-rectangular (NRH) and rectangular (RH) hyperbolas and the exponential (EXP) models were compared. All three models were quantitatively adequate to describe the irradiance response of photosynthesis in dwarf coconut. The photosynthetic parameters showed good correlation with the measured values when estimated by the rectangular hyperbola and exponential but the former overestimated the light-saturated rate and the apparent quantum yield of photosynthesis. The NRH was less adequate for estimate the light-saturated photosynthesis and dark respiration universally, but could be used for research purposes since it contains an important qualitative parameter, the convexity term relating physical to total resistances to CO_2 diffusion into the chloroplasts. The results allow suggesting the exponential model as a rapid, simple, quantitatively and qualitatively adequate option for accessing information from the photosynthetic irradiance-response in dwarf coconut palm.

*Key words***:** apparent quantum yield, compensation irradiance, convexity term, dark respiration, light-saturated rate of net photosynthesis.

Resumo Dados experimentais de curvas de resposta da fotossíntese à irradiância, obtidos em experimentos independentes com o coqueiro Anão-Verde do Brasil procedente de Uma, Bahia (UGD), foram utilizados para ajustar três modelos empíricos com o objetivo de comparar suas performances quantitativa e qualitativa. Os modelos comparados foram as hipérboles retangular (RH) e não-retangular (NRH) e o modelo exponencial (EXP). Os resultados mostraram que os três modelos foram considerados quantitativamente adequados. Os parâmetros fotossintéticos mostraram estreita correlação com os valores medidos, quando estimados por RH e EXP. Entretanto, a RH superestimou a taxa fotossintética máxima (saturada por irradiância) e a eficiência quântica aparente. A NRH foi menos adequada para estimar os parâmetros fotossintéticos universalmente, mas pode ser utilizada para a pesquisa, visto que apresenta como vantagem qualitativa a presença do termo de convexidade que representa a razão entre as resistências física e total à difusão de CO_2 para os cloroplastos. Os resultados permitem sugerir o modelo exponencial como uma opção simples, rápida, quantitativa e qualitativamente adequada para acessar as informações das curvas de resposta de *A* a irradiância.

1. Introduction

Complex mechanistic models of photosynthesis such the biochemical model of Farquhar et al., (1980) for C_3 leaves, has been adopted in studies of ecological and physiological modeling, since it is capable of describing underlying processes that might not be well described by empirical models. However, it requires rather extensive calibration of a number of parameter as well as complex parameterization procedures (Cannell and Thornley, 1998). Moreover, detailed treatment of biochemical processes in biochemical photosynthesis models not always is advantageous over the simpler leaf photosynthesis models (Gao et al., 2004). Nevertheless, empirical models have been used extensively in ecophysiology for accessing and exploring information derived from irradiance-response curves of photosynthesis. For example, it has been used in studies of the effects of biotic (Habermann et al., 2003) and abiotic stresses such as drought (Escalona et al., 1999), salinity (Krauss and Allen, 2003), soil flooding (Mielke et al., 2003) and temperature (Peri et al., 2005), since they have simple formulas, can be easily parameterized and their parameters have a straightforward interpretation. The objective of the present study was to compare the three traditional models (rectangular and non-rectangular hyperbolas and exponential) for describing the photosynthetic irradiance-response curve using data from dwarf coconut palm. We tested the hypothesis that the three models are quantitatively adequate and that they behave distinctly when used for estimating the main photosynthetic parameters.

2. Material and Methods

2.1. Sites and plant material

The analyses were performed using the photosynthetic irradiance-response curves measured in adult and young plants of Brazilian Green Dwarf coconut (*Cocos nucifera* L. *'nana'*), ecotype from Una, Bahia (UGD) grown under field and greenhouse conditions, respectively. The sites (1 to 4) of data collection in the field were described in Gomes et al. (2002). The sites 1 to 3 are located at the southeast region of Bahia State, Brazil, including an intercrop system with cocoa (*Theobroma cacao* L.), cupuaçu (*T. grandiflorum* L.) and plants of Brazilian green dwarf coconut (*Cocos nucifera* var. *nana* L., Palmae) as main culture, located in the Cocoa Research Center Experimental Station, in the municipality of Una (15º15'45"S, 39º05'49"W, 105 masl) (site 1), a private farm in the municipality of Ilhéus (14º46'52"S, 39º14'38"W, 15 masl) (site 2) and a private farm in the municipality of Canavieiras (15º38'27"S, 39º07'04"W, 49 masl) (site 3). Site 4, a constrasting environment, is a private farm located at the San Francisco river valley, municipality of Petrolina, Pernambuco State (09º27'42"S, 40º 57'0"W, 376 masl). The greenhouse experiment (site 5) was conducted at the Plant Growth Center of the Federal University of Viçosa, Brazil (20º 45'S, 42º 52'W, 648m asl), where potted seedlings of the BGD coconut genotype were grown for 7 months. At the moment of measurements the plants had 1.2 m and 5-7 expanded leaves. Intact leaves of rank 2 and 9 (counted from the top down) were used for the gas exchange measurements in young and adult palms, respectively. A total of 32 irradiance-response curves were used in the analysis: 3 from site 1, 9 from site 2, 11 from site 3, 3 from site 4 and 6 from site 5.

2.2. Photosynthetic measurements

The measurements were performed always from 7:30h to 11:00h using a Portable Photosynthesis System LI-6400 (Li-Cor, USA) equipped with an artificial irradiance source 6400-02B RedBlue. It used the "light curve" routine of the software OPEN 3.4, in seven levels of photosynthetic photon flux density (*PPFD,* 50, 100, 200, 400, 800, 1200 and 1600 µmol photons m^{-2} s^{-1}) in a decreasing order. The minimum time allowed for the reading stabilization at each level of *PPFD* was 120s, and the maximum time for saving each reading was 150s. It was set a flow rate of 400 µmol s^{-1} and a maximum coefficient of variation (C.V.) of 1%. The values of atmospheric CO_2 concentration and of leaf-to-air water vapour pressure deficit inside the assimilation chamber were 376 ± 11 µmol mol^{-1}, 1.4 ± 0.5 kPa, respectively. Mean leaf temperature was 28.6 ± 2.2ºC and increased 0.8ºC on average with increasing irradiance, depending on the site.

2.3. Description of the models

Three empirical models were tested:

Non-rectangular hyperbola, NRH (Marshall and Biscoe, 1980)

$$A_n = \{[A_{max} + (\alpha\, PPFD) - [(A_{max} + (\alpha\, PPFD))^2 - (4\alpha\, q\ \mathrm{PPFD}\, A_{max})]^{0.5}] / (2q)\} - R_d + \varepsilon$$

Rectangular hyperbola, RH (Thornley, 1976)

$$A_n = \{(A_{max}\, \alpha\, PPFD) / [A_{max} + (\alpha\, PPFD)]\} - R_d + \varepsilon$$

Exponential, EXP (Iqbal et al., 1997)

$$A_n = \{A_{max}\, [1 - \exp((-\alpha\, PPFD) / A_{max})]\} - R_d + \varepsilon$$

where A_n is the rate of net photosynthesis, µmol CO_2 m^{-2} s^{-1}; A_{max} is the irradiance-saturated rate of gross photosynthesis, µmol CO_2 m^{-2} s^{-1} ($A_{max} = A_{nmax} + R_d$, where A_{nmax} is the irradiance-saturated A_n and R_d is the dark respiration rate, µmol CO_2 m^{-2} s^{-1}, corresponding to the value of A_n when $PPFD = 0$ µmol photons m^{-2} s^{-1}); α is apparent quantum yield of photosynthesis; q is a dimensionless convexity term; and ε is the random error. For correlation analysis between measured and estimated values, “measured” α was estimated by the linear regression of A_n to $PPFD < 200$ µmol m^{-2} s^{-1} (Escalona et al., 1999). In the linear equation α is the coefficient of the independent term (slope of the curve). Measured A_{nmax} is the mean value of A_n when $PPFD > 800$ µmol photons m^{-2} s^{-1}.

2.4. Statistical analysis

Every model was fitted to measured data from each one of the 32 irradiance-response curves using the Levemberg-Marquardt algorithm of the non-linear least squares estimate routine of the software Statistica 6.0 (StatSoft, Inc). The initial values for A_{max}, α, R_d and q were set at 5 µmol CO_2 m^{-2} s^{-1}, 0.01 mol CO_2 mol^{-1} photons, 0.1 µmol CO_2 m^{-2} s^{-1} and 0.1, respectively, according to the coherence of the predicted values. The adequacy (goodness-of-fit) of the models was verified by plotting the modeled curve for all data with the mean measured values for each site, by the analysis of residuals i.e., the deviations between the measured and predicted values, and by a special *F*-test (Pachepsky et al., 1996). Correlation analyses between predicted and measured (or linearly estimated) values were also performed and the r^2 was used as an accessory indicator for the validation of the models.

3. Results

All three models showed a good fit ($r_{\{y} > 0.95$) to the experimental data with good correspondence with the shapes of the mean measured values at all sites (Fig. 1). Goodness-of-fit was confirmed for all sites by the *F*-test, indicating that the three models were quantitatively adequate, except for the RH at site 5 (Table 1).

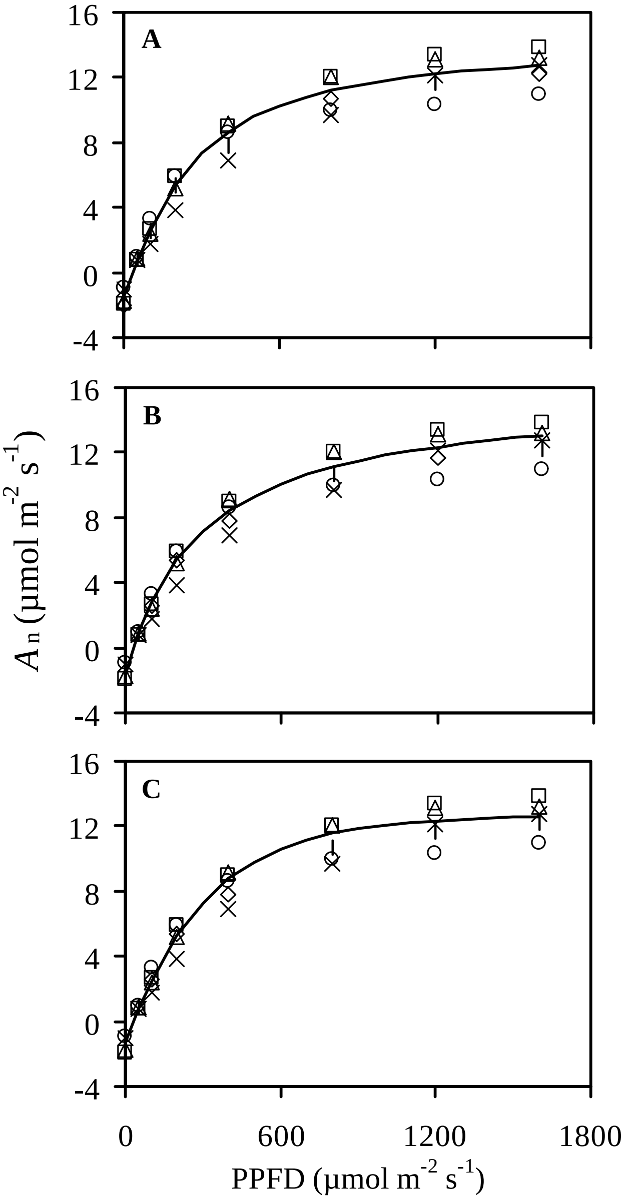

Fig. 1: Irradiance-response curves of net photosynthesis (A_n) in dwarf coconut. The points are the measured mean values of A_n at sites 1 (◊), 2 (Δ), 3 (ð), 4 (x) and 5 (o) and the lines represent the non-rectangular hyperbola (A), the rectangular hyperbola (B) and the exponential (C) models.

Table 1: *F*-test (p ≤ 0.05) for accessing the quantitative performances of the non-rectangular (NRH) and rectangular (RH) hyperbolas and the exponential (EXP) models describing the irradiance-response of photosynthesis in dwarf coconut. *, indicate significance ($P \leq 0.05$)

Model	**Site**	***F***	***F* (tab)**
NRH	1	1.89	3.06
	2	0.48	2.56
	3	0.30	2.49
	4	0.11	3.05
	5	1.56	2.61
	All	1.42	4.12
RH	1	2.05	2.90
	2	1.92	2.37
	3	0.20	2.33
	4	0.07	2.9
	5	3.53*	2.45
	All	1.47	3.27
EXP	1	1.93	2.90
	2	0.36	2.37
	3	0.80	2.33
	4	0.16	2.9
	5	1.29	2.45
	All	1.17	3.27

On the other hand, the absolute value of *F* for the NRH and RH were larger than that for EXP. The RH and EXP models showed a random distribution of the residuals around the predicted values at all sites (Fig. 2). Under high irradiance, the NRH showed a clear underestimation of A_n at the site 1 and overestimation at the sites 2 and 5 (Fig. 2).

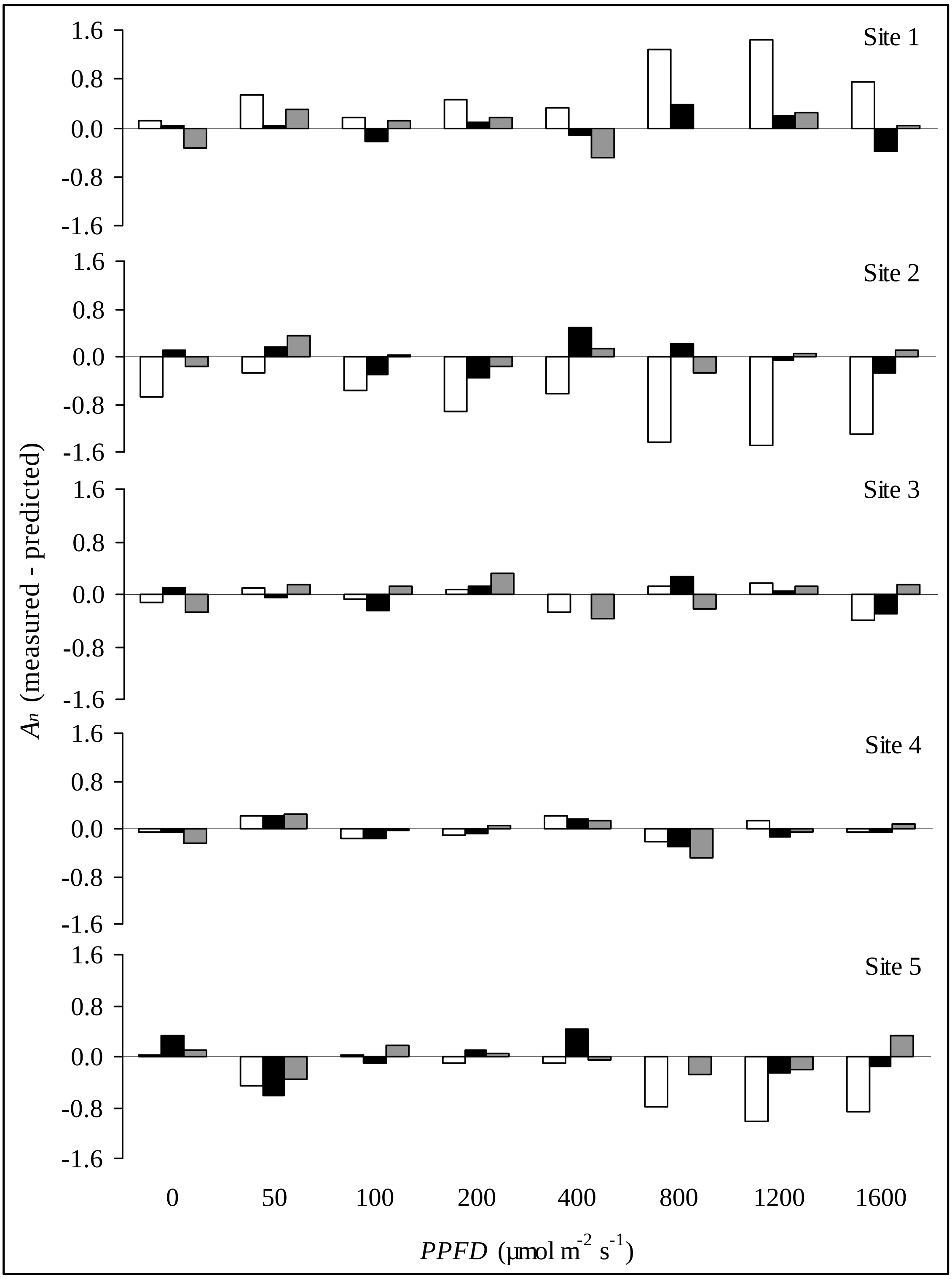

Fig. 2: Measured minus predicted values of net photosynthesis (A_n) obtained after adjusting the non-rectangular hyperbola (white), the rectangular hyperbola (black) and the exponential (gray) models to the field data of irradiance-response curves of photosynthesis in dwarf coconut at the five sites.

A linear trend was observed between measured and predicted values of A_{nmax} by the RH ($r^2 = 0.80 – 0.95$, Fig. 3B) but the values predicted by the NRH showed a large scatter with poor linear correlation at sites 2, 3 and 5 (Fig. 3A). The two hyperbolas estimated the higher values of A_{nmax} as compared to measured light-saturated A. While predicted and measured R_{d} showed good correlations at all sites when estimated by the RH (Fig. 3E, $r^2 = 0.89$) and EXP (Fig. 3F, $r^2 = 0.83$), this parameter was lower than the measured R_{d} at site 2 and higher at site 4 when estimates by the NRH (Fig. 3D). Comparing α estimated by the non-linear models (predicted) with that estimated by linear regression (quoted as measured), it was observed that all three models showed good linear correlation with mean r^2 varying from 0.69 to 0.71 (Fig. 3G, H), but, like A_{nmax}, when predicted by the two hyperbolas, α was higher than the values estimated by linear regression. The three parameters were better estimated by the exponential model, whose outputs were linearly correlated with measured values (Fig. 3C, F, I).

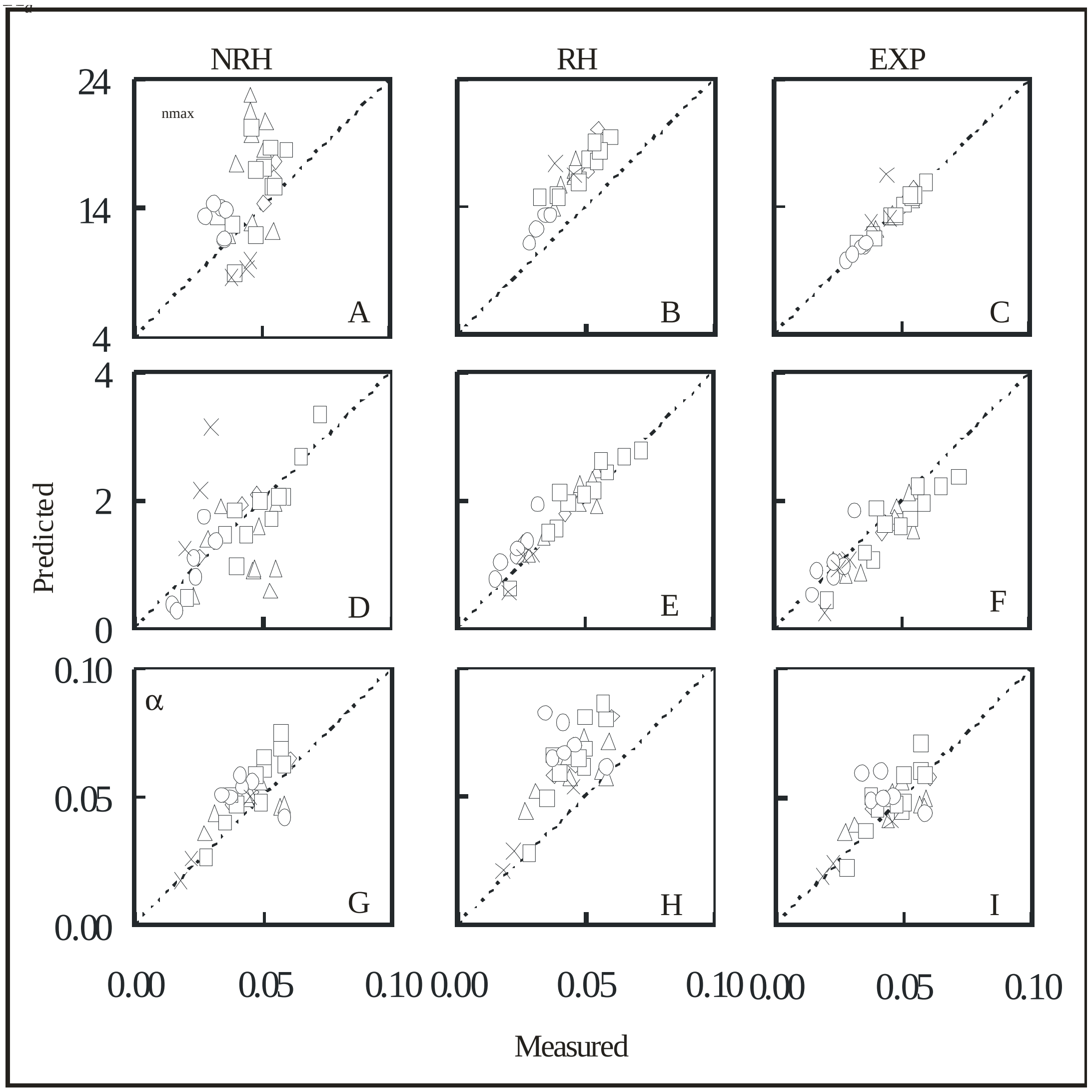

Fig. 3: Predicted and measured values of the irradiance-saturated net photosynthesis (A_{nmax}, A, B, C), dark respiration (R_d, D, E, F) and apparent quantum yield (α, G, H, I) in dwarf coconut. NRH, non-rectangular hyperbola; RH, rectangular hyperbola; EXP, exponential. Sites 1 (◊), 2 (Δ), 3 (ð), 4 (x) and 5 (o). The 1:1 lines (dotted) are shown.

4. Discussion

The special *F*-test (Pachepsky et al., 1996) allowed comparison of the variability of predictions (lack-of-fit mean square) with the variability of data (pure error mean square). It must be noted that the denominator of *F* (data variability) is the same for all three models so the numerator characterizes the lack-of-fit of the model. The lower absolute value of *F* for EXP may indicate a slight better quantitative performance of this model when compared to the hyperbolas. On the other hand, whereas the NRH model was considered quantitatively adequate by the *F* test (Table 1), it was shown to be inadequate for two of five sites by the residual analysis (Fig. 2).

The RH gave higher A_{nmax} and α, when compared to measured (or linearly estimated) values, as has been observed in other studies (Lamade and Setiyo, 1996; Mielke et al., 2003). Nevertheless, this model was quantitatively adequate for dwarf coconut and could be used as a submodel for predictive calculations in productivity models. Dufrene et al. (1990) used a RH as component of a complex model to estimate canopy assimilation, which allowed understanding the links between productivity and climatic factors in oil palm. Moreover, high predicted α as compared to the linearly estimated values (Fig. 3) could be due, in part, to the use of a linearly underestimated α for comparison. Using linear regression to estimate α of modeled light curves with q = 0.89, Leverenz et al. (1990) observed an underestimation of α by about 9%.

The EXP model was also quantitatively adequate to describe the photosynthetic irradiance response in dwarf coconut and provided a more accurate and realistic estimation of the photosynthetic parameters when compared to the two hyperbolic models. Like the RH it has few and easily interpreted parameters and could be used for predictive purposes either isolated or as part of nonlinear mixed models (Peek et al., 2002).

It was demonstrated here that NRH was less adequate for A_{nmax} and R_{d} predictions at all sites, which suggest its limited applicability for predictive purposes. Nevertheless, this model has been preferred for research purposes either alone or as a part of a larger model to study the mechanisms of photosynthesis (Peri et al., 2005). A mean value of 0.50 for its convexity term (q), as estimated in the present work (data not shown), was interpreted to be a result of a low physical limitation (high stomatal conductance) as compared to internal (mesophyll and biochemical) resistances to CO_2 diffusion into the chloroplast (Marshal and Biscoe, 1980). Although more specific experiments are necessary to strengthen that interpretation, high values of stomatal conductance (0.3 to 0.7 mol H_2O m^{-2} s^{-1}) usually measured in dwarf coconut varieties (Passos et al., 1999; Gomes et al., 2002) corroborate the present results. On the other hand, the value of 0.50, in spite of being considered low when compared to other C_3 species (Ögren, 1993), could be due to the adaptation of coconut to high light environments. According to Leverenz (1994), as the mismatch between the gradients of A_{max} and of *PPFD* through the leaf increases, the value of q decreases more from its intrinsic value in the chloroplasts so that high irradiance-adapted plants may show very low q, depending upon the species. Thomas and Bazzaz (1999) estimated values of q ranging from 0.20 to 0.80 for tree species of dipterocarp forest of the Indo-Malayan region.

The results allow concluding that all three models were quantitatively adequate for fitting experimental data of irradiance-response curves in dwarf coconut palm but A_{nmax} and α were overestimated by the two hyperbolas. The NRH was less adequate for estimate A_{nmax} and R_d universally, but could be used for research purposes since it contains an important qualitative parameter, the convexity term. The use of exponential models for accessing the information of the photosynthetic irradiance-response curves of Brazilian Green Dwarf coconut is recommended.

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CONCLUSÕES GERAIS

Plantas jovens de dois ecotipos do cultivar Anão-verde do Brasil foram submetidas a três ciclos sucessivos de deficiência hídrica. Os resultados mostraram que as variáveis de trocas gasosas (*A*, *gs* e *E*) decresceram após a suspensão da irrigação e atingiram valores próximos a zero quando o potencial hídrico (Ψ_{PD}) decresceu a -1.2 MPa, sugerindo elevada sensibilidade dessas variáveis à deficiência hídrica no solo. A relação entre *A* e *gs* foi exponencial, indicando que as limitações estomáticas à assimilação de carbono foram observadas em $gs < 0.2$ mol m^{-2} s^{-1}. Acima desse valor, *A* praticamente não variou enquanto a eficiência intrínseca de uso da água (*A*/*gs*) praticamente dobrou em relação aos valores observados nas plantas controle. Esses resultados sugerem que é possível manipular a condutância estomática do coqueiro anão sem prejuízos para a assimilação de carbono, mas com ganhos expressivos para economia hídrica da planta.

Demonstrou-se, pela primeira vez em coqueiro, que a concentração de ácido abscísico aumentou nas folhas em resposta à redução do teor de água no solo. Além disso, o fechamento estomático em coqueiro anão sob estresse moderado foi relacionado mais aos aumentos na concentração foliar de ABA do que às alterações no status hídrico da folha.

Reduções significativas no potencial osmótico foram observadas em resposta aos ciclos de deficiência hídrica. Entretanto, observou-se reduzida capacidade de ajuste osmótico, o qual foi limitado quanto à manutenção do turgor celular sob condições de seca severa, visto que se observou redução severa no crescimento das plantas. Além disso, se os custos energéticos envolvido na biossíntese, transporte e compartimentação dos solutos forem levados em consideração, percebe-se que o ajuste osmótico pode ter agravado o distúrbio metabólico desencadeado pela deficiência hídrica.

A concentração foliar de prolina praticamente dobrou nas plantas estressadas durante a deficiência hídrica, apesar desse acúmulo não ter sido relacionado ao ajustamento osmótico. Sugere-se que o acúmulo de prolina em coqueiro anão esteja mais relacionado a funções osmoprotetivas do que osmoregulatórias.

Sob deficiência hídrica severa, caracterizada, nas condições do presente experimento, por $gs < 0.04$ mol m^{-2} s^{-1} e $\Psi_{PD} < -1.0$ MPa, fatores não estomáticos passaram a ser importantes, os quais contribuíram para a limitação de A tanto durante o estresse severo quanto durante a recuperação nos dois ecotipos. As limitações não-estomáticas foram caracterizadas no presente estudo por reduções significativas na taxa fotossintética saturada por CO_2 (A_{maxCO2}), na eficiência de carboxilação (CE), na taxa máxima de transporte de elétrons além da ocorrência de fotoinibição.

Entre os modelos empíricos frequentemente utilizados para descrever a resposta de A a radiação fotossinteticamente ativa (hipérboles retangular e não-retangular e exponencial) demonstrou-se que, embora os três modelos fossem quantitativamente adequados para a estimativa dos parâmetros fotossintéticos, o exponencial foi o que melhor se comportou quanto à fidelidade das estimativas, sendo, portanto, recomendado.

Os ecotipos de coqueiro anão-verde utilizados no presente trabalho mostraram baixa tolerância à deficiência hídrica, mostrando reduções expressivas na taxa de assimilação de carbono e no crescimento, apesar de mostrar características de evite da desidratação por meio de fechamento estomático precoce sob condições estressantes. Entretanto, o Anão-Verde de Jiqui mostrou algumas vantagens quando comparado ao Anão-Verde de Una como maior eficiência de uso da água ao final do experimento e maior capacidade de acúmulo de prolina. Além disso, durante o período de recuperação, o Anão-Verde de Jiqui mostrou maior eficiência quântica aparente e sinais de aclimatação da capacidade fotossintética (menor efeito da seca em A_{maxCO2}, CE e na concentração de pigmentos foliares,) após sucessivos ciclos de deficiência hídrica.