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Photochemical energy use in Sorghum plants grown under drought and elevated CO_2.

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Introduction

Decreased CO_2 assimilation induced by water-stress, diurnal fluctuations in light intensity, temperature and leaf-to-air vapor pressure deficits (VPD) can lead to over excitation of the photosynthetic apparatus inhibiting photosynthesis and reducing crop productivity. The absorption of a photon of light by the light harvest complexes can return to the ground state by either transfer of the excitation energy to a reaction center to drive photochemistry, be remitted as Chl fluorescence, decay via the triplet state or by thermal dissipation as non-photochemical quenching (NPQ). The pH-dependent (qE) energy dissipation is the most significant component of NPQ and is primarily controlled by the thylakoid ΔpH and a decreased lumenal pH generated during electron transport. Additionally, qE and low lumenal pH are strongly correlated with the conversion of vioaxanthin to zeaxanthin during the xanthophyll cycle (Demmig-Adams, 1990). Our study was conducted to examine how growth under elevated CO_2 and water-stress conditions affects PSII photochemistry, carbon assimilation (A) and photoprotection in Sorghum plants grown under a FACE experiment. We have further characterized the effect of water-stress and CO_2 availability on the relationship of NPQ and the xanthophyll cycle under laboratory and field conditions.

Materials and methods

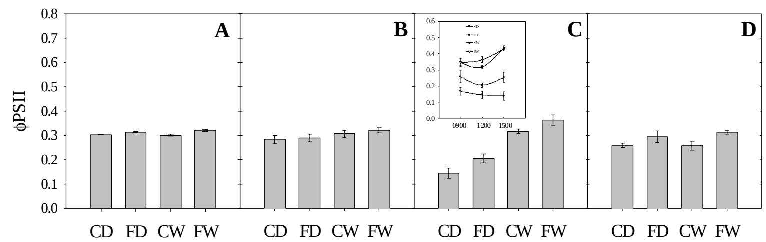
Free-air CO_2 enrichment (FACE) experiments were conducted at the University of Arizona Maricopa Agricultural Center (MAC), Maricopa, AZ, USA 1999 to determine the interactive effects of elevated CO_2 and drought on *Sorghum bicolor* (L.). The FACE plots were enriched to a target 200 µL L^{-1} above Control and each plot was split, with each half receiving either an ample (wet) or a water-limited (dry) irrigation regime.

A modulated chlorophyll fluorimeter and leaf clip (PAM 2000, Walz) was used to measure variation in Fo′, Fm′ and Fs. The apparent quantum yield of Photosystem II, ϕPSII (Fm′-Fs/Fm′) was determined as described by Genty *et al*., (1989). Fv/Fm was measured following dark adaptation for 15 min. Non-photochemical quenching (NPQ) was determined as (Fm-Fm′)/Fm′. Pigment extracts were fractionated by HPLC and identified by retention time and spectrophotomertic properties. On 08/06/99 excised uppermost fully expanded leaf were placed into a 6400-06 PAM2000 adapter cuvette (LiCor, Inc. Lincoln, NE). Steady state photosynthetic measurements (~800 µmol photon m^{-2} s^{-1}) were made at several CO_2

concentrations in air containing 21% O_2 and then 2% O_2. Leaf tissue was immediately freeze-clamped and stored in liquid nitrogen until pigment and biochemical assays were conducted.

Results

Midday rates ϕPSII were significantly enhanced by growth under elevated CO_2 ($F_{1, 3.09} = 15.6$**). Water treatment was significantly dependent on DAP ($F_{3, 35.5} = 30.55$***) and two-ANOVAs for each sampling data indicated that the differential water treatment reduced midday levels of ϕPSII ($p \leq 0.001$) only on 08/04/99 (Fig.1 a-d). In the drys elevated CO_2 significantly enhanced ϕPSII over the course the day, but only at midday in the wets (inset Fig.1c). On 08/04/99 midday levels of Fv/Fm showed no treatment effect (not shown). NPQ was greater in the water-stressed plants then in the well-watered plants but elevated CO_2 reduced rates of NPQ in both water treatments (Fig.1e). Midday field samples showed high levels (~40%) of the de-epoxidation state of the xanthophyll pool, DPS (Z+A/V+A+Z), however, there were no significant differences between treatments (Fig.1f).



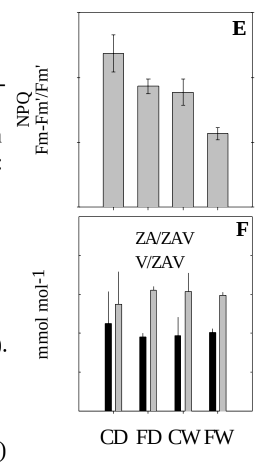


Figure 1. Mean midday ϕPSII on sun lit upper most fully expanded leaves (±1SE) n=4 (a-d). Three-way ANOVA tested effect of CO_2, H_2O and DAP on ϕPSII. F values and significance: CO_2 ($F_{1, 3.09} = 15.6$**), H_2O ($F_{1, 5.63} = 51.39$***), DAP ($F_{3, 35.5} = 11.9$***) and interactions DAP*CO_2 ($F_{3, 35.5} = 2.23^{ns}$), DAP*H_2O ($F_{3, 35.5} = 30.55$***), CO_2*H_2O ($F_{1,5.63} = 0.07^{ns}$), DAP*CO_2*H_2O ($F_{3, 35.5} = 0.34^{ns}$). ns not significant; ** $P \leq 0.05$; *** $P \leq 0.01$. Diurnal ϕPSII on 08/04/99 (inset, Fig.1c). Non-photochemical quenching (NPQ) and xanthophyll de-epoxidation state on 08/04/99 (Fig.1e & f respectively).

On 08/06/99 the upper most fully expanded leaves were excised from plants removed from the field at predawn. Rates of carbon assimilation were significantly enhanced by elevated CO_2 whereas ϕPSII measured at growth CO_2 concentrations showed no major differences between plants from the different growth treatments. In the plants grown in the water stress treatment there was no differences in the rates of NPQ when the drought conditions were alleviated; however, NPQ measured at growth CO_2 concentrations was reduced by elevated

CO_2 in both water treatments (Table 1). Pigment analysis on the same leaf tissue showed no CO_2 effect, however, the DPS of the xanthophylls was approximately 57% higher ($p \leq 0.01$) in the dry versus the wet plants. Regression analysis of ϕPSII against ϕCO_2, measured at various Ca, on mature leaves showed no significant effect of elevated CO_2 or water treatment on either the slope or the intercept. The total Rubisco activity to total activity PEPC was 17% lower in the water stressed plants due to increased activity of PEPC and unchanged levels of Rubisco but unaffected by CO_2 treatment (Table 1).

Table 1. Mean (SE) A, ϕPSII, NPQ, carotenoid composition, Rubisco and Pepc full activities measured under laboratory conditions on 08/06/99. Measurements were made at growth CO_2 concentrations on the upper most fully expanded leaves excised under water. The effects of CO_2 ($F_{1,4}$)and water treatment ($F_{1,2}$) and their interaction ($F_{1,4}$) were tested for significance by two way ANOVA. *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.1$, ns not significant.

Parameters	DRY		WET		ANOVA		
	control	FACE	control	FACE	CO_2	H_2O	CO_2*H_2O
A $\mu mol\ CO_2\ m^{-2}\ s^{-1}$	22.6 (3.6)	25.6 (3.7)	27.4 (1.3)	31.1 (1.5)	*	ns	ns
ϕPSII	0.31 (0.07)	0.37 (0.04)	0.34 (0.05)	0.42 (0.02)	ns	ns	ns
NPQ	2.6 (0.5)	1.6 (0.3)	2.3 (0.2)	1.5 (0.1)	***	ns	ns
ZA/ZAV $mmol\ mmol^{-1}$	0.35 (0.07)	0.44 (0.09)	0.16 (0.06)	0.28 (0.1)	ns	*	ns
Rubisco $\mu mol\ CO_2\ m^{-2}\ s^{-1}$	23.9 (4.0)	20.2 (2.7)	17.7 (1.8)	18.5 (0.9)	ns	ns	ns
Pepc $\mu mol\ CO_2\ m^{-2}\ s^{-1}$	530 (33)	450 (69)	366 (5.0)	361.7 (42)	ns	***	ns

Discussion

PSII photochemistry

Midday levels of ϕPSII was higher in plants grown under elevated CO_2 regardless of water treatment, suggesting that elevated CO_2 enhances photosynthesis in Sorghum leaves even in well-watered plants. The relatively moderate midday CO_2 enhancement of photosynthesis in the wet plots was not great enough to significantly increase plant growth or yield. However, during water stress conditions, the large diurnal enhancement of photosynthesis by growth under elevated CO_2 likely contributed to the increased crop yield seen in the FACE-dry plants compared to the Control-dry plants.

Photoprotection

The fact that rates of photosynthesis recovered to prestressed levels upon reirrigation indicates that limited CO_2 fixation and high light associated with drought conditions did not have a long-term effect on the photosynthetic apparatus. Additionally, prior water treatments had no effect on the rates of ϕPSII, Fv/Fm or A measured under non-stress conditions on leaves of plants removed from the field on 08/06/99. ϕPSII and ϕCO_2 measured simultaneously at various levels of CO_2 in a control laboratory environment maintained a constant relationship. This suggests that in mature Sorghum leaves A is the primary electron sink and photorespiration and/or electron flux to O_2 has a minimal role in photoprotection even at low [Ci]. Reduced levels of ϕPSII in the field were primarily attributed to increased rates of NPQ as well as an increased reduction state of PSII. Drought conditions resulted in an increase in the total xanthophyll cycle pool size, however, under midday field conditions there was no significant treatment effect on the DPS. This lack of treatment effect on the DPS is inconsistent with the large differences in NPQ seen in the same leaves. Similar to the field conditions NPQ was lower under elevated CO_2 and DPS was not responsive to CO_2 treatments, however, DPS was significantly higher in the plants removed from the water-stress treatment. It has been recently suggested that the lumenal pH has an additional role in regulating NPQ in addition to the xanthophyll cycle (Muller *et al.*, 2001). It is possible that increased photosynthetic rates under elevated CO_2 alleviated ΔpH sufficient to reduce rates of NPQ but was insufficient to reverse the xanthophyll cycle in the direction of violaxanthin.

Rubisco and PEPC activity.

Our experiment with mature FACE grown plants showed no CO_2 effect on the total amount of Rubisco and PEPC activity per leaf area (Table 1). However, drought conditions significantly decreased the ratio of Rubisco to PEPC as previously seen in other experiments. Under field conditions, increases of atmospheric CO_2 to levels predicted to occur within the next 50 years (ambient +200ppm) was insufficient to alter key components of the C_3 and C_4 cycle even when atmospheric CO_2 availability was further limited by drought conditions. It is likely that changes in C4 photosynthetic enzymes will be more sensitive to climatic changes other then increased Ca.

Conclusions

Growth under elevated CO_2 enhanced C_4 photosynthesis when carbon assimilation was limited during drought and/or midday conditions. Midday rates of ϕPSII in Sorghum leaves were higher under elevated CO_2 in both water treatments, however, midmorning and late afternoon rates of ϕPSII were increased only in the dry treatment. NPQ was higher in the water-stressed plants but was reduced by elevated CO_2 in both water treatments. Additionally, NPQ and pigment compositions were affected differently by elevated CO_2 and water-stress. Low NPQ under high CO_2 did not correlate with DPS which maybe attributed to a reduced thylakoid ΔpH sufficient to alleviate NPQ but not DPS. The enhanced diurnal rates of ϕPSII in the FACE-dry treatment likely contributed to the enhanced grain and total yields relative to the Control-dry treatment.

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