Title: Diurnal regulation of photosynthesis in *Jatropha curcas* under drought during summer in a semi-arid region

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Abstract

The diurnal photosynthetic responses to drought in *Jatropha curcas* have not been well assessed under field conditions in harsh semi-arid habitats. To illustrate this, diurnal changes in chlorophyll fluorescence and gas exchange rates were measured in field-grown *Jatropha* with or without a short (13 days) water recovery treatment under drought conditions during hot summer in a semi-arid. Sensitive stomatal closure coordinated with a drying atmosphere strictly limited a net CO₂ assimilation rate with a predominant morning peak, eventually turning negative during the day. Even though the risk of excess excitation energy which potentially causes photodamage increased with the extremely low capacity for CO₂ fixation, *Jatropha* preserved the integrity of PSII. Quantitative analysis of quenching partitioning revealed that regulated thermal energy dissipation accounted a large fraction of both instantaneous and daily absorbed energy by up to 80 and 72 %, respectively, under the drought condition. Water recovery treatment more than doubled daily CO₂ uptake via mitigating diurnal stomatal closure. The regulated thermal dissipation flexibly adjusted PSII quantum efficiency to capacity of CO₂ fixation. In addition, downregulation of PSII quantum efficiency via sustained regulated thermal dissipation was observed and thought to be an additional photoprotective function. It is clear that *Jatropha* strongly rely upon the regulated thermal dissipation under drought condition, which must be critically important for this strict water conserving species, especially under a climate with high solar radiation loads as is seen in semi-arid regions.

Key words: Drought stress, Diurnal photosynthesis, Quenching partitioning, Non-photochemical quenching, Thermal dissipation, Semi-arid
1. Introduction

*Jatropha curcas* is a soft-woody plant of the Euphorbiaceae family that produces rich oilseeds and has recently attracted worldwide attention as a source of biodiesel [1]. It originated from Meso-America, but has begun to be cultivated around the world, including in semi-arid regions in Africa [2,3]. However, little is known about the productivity and adaptability of *Jatropha* in semi-arid climate conditions, restricting our capacity to properly evaluate and predict the agronomic performance of this species. This is surprising, especially when we consider that one of its great advantages as a potential crop is its ability to grow in semi-arid marginal agricultural land without competing with food production or environmental conservation [1,4].

Photosynthesis is the primary source of plant production and is also among the primary processes to be affected by drought [5,6]. Recently, increasing number of studies investigate photosynthetic response to drought in *Jatropha*. Strict stomatal regulation in *Jatropha* under water deficit has been reported in previous studies [7-11]. Acclimation of osmotic adjustment by means of increases in inorganic [10] and organic solutes [12] and of increased activities of several antioxidant enzymes [8,9] to prolonged water stress coordinated with a decrease in photosynthetic rate ($A$) have also been observed. Some studies applied simultaneous measurements of chlorophyll fluorescence and gas-exchange rates to *Jatropha* leaves. The progressive breakage of the link between the electron transport rate and CO$_2$ assimilation rate were found under drought in *Jatropha* [9], indicating that the alternative electron flow mediates the antioxidant activity in water-stressed *Jatropha* leaves. Chronic photoinhibition, indicated by decreases in predawn $F_v/F_m$ [13], is rarely seen under moderate to severe drought even when the photosynthetic rate is strongly impaired [9,10,14,15]. Instead, the increase in non-photochemical quenching (NPQ), namely rapidly reversible components of thermal dissipation [16], under the water deficit as well as downregulation of PSII quantum efficiency ($\Phi_{PSII}$) were reported and thought to have photoprotective functions via dissipation of excess energy at the PSII [9,15]. These different levels of characteristic responses to water stress in *Jatropha* however, have been identified under controlled, relatively mild climatic conditions with pot experiments, i.e., relatively low light intensity and moderate temperature and humidity. In semi-arid climates, such as in the
Mediterranean region, where a scarcity of precipitation generally coincides with sunny days, water stress on photosynthesis might be further exacerbated by the excess solar radiation loads and other co-factors, such as high temperatures and evaporative water demand [17]. In addition, plants in arid habitats are known to conserve water by means of midday stomatal closure [18], which is often either excluded or discounted in a controlled experiment. Accordingly, it is difficult to assess the relative importance of these photosynthetic responses to drought without understanding the field performance under drought condition. However, a few studies have assessed diurnal photosynthesis in *Jatropha* grown in semi-arid where plants were exposed to water deficits with multiple stress factors.

Quenching partitioning through light-modulated chlorophyll fluorescence can quantitatively assess the fate of the absorbed light [16,19,20,21]. It has been demonstrated that quenching partitioning is useful approach for evaluating relative importance of NPQ to photoprotection by estimating both instantaneous and daily cumulative fraction of excitation energy that is dissipated via the various pathways involving photochemical and non-photochemical quenching in the field condition [19,20]. Thus, applying the quantitative analysis of quenching partitioning can be supplemental for evaluating in situ diurnal photosynthetic response to potentially excess photons under stress conditions.

The objective of this study is to investigate the regulation of diurnal photosynthesis in *Jatropha* under drought conditions in a harsh semi-arid habitats, especially focusing on the fate of the absorbed light with quantitative analysis of quenching partitioning, as well as to assess the effects of water stress on the regulatory processes. The effects of water stress were investigated in comparison with a water recovery treatment under the field condition.
2. Materials and methods

2.1. Experimental site

The experimental site was an agricultural field located in the Department of Agricultural Research, Ministry of Agriculture of Botswana (25°56′37″E, 24°33′40″S, 992 m a.s.l.). The area is categorized as a semi-arid climate [22], with a wide range of diurnal temperatures throughout the year. In the summer season, the lowest temperature reaches below 15°C in the morning whereas the highest temperature reaches over 40°C at midday. Mean annual precipitation is less than 490 mm on average, which is lower than what is considered the optimal value of between 1000 and 1500 mm for the growth of Jatropha [4]. The precipitation in 2012 at the experimental site was only 333 mm. Rainfall mainly occurs during October and March, accounting for almost 100% of the annual rainfall. The reddish-brown soil is categorized as a Rendzic Leptosol, with poor fertility, high aluminum and iron, and mainly consisting of silt and clay (personal communication). Healthy Jatropha seedlings from the seeds of Botswana accessions were transplanted into the 0.5 ha field with a spacing of 2 m × 2 m in Dec 2011. Manual irrigation was performed from Dec 2011 until July 2012, and a drip irrigation system was installed in July 2012 that provided 5 L week⁻¹ of water per plant during the rest of the dry season until Oct 2012. From Nov 2012, the irrigation stopped and the only water supply was from rain. A pruning treatment was done in May 2012 for overcoming winter coldness, and 90% of the Jatropha plants survived through the winter in 2012.

2.2. Diurnal gas exchange and chlorophyll fluorescence

In situ gas-exchange and fluorescence parameters were measured in Jatropha with a portable photosynthesis system equipped with a 6400-40 leaf chamber fluorometer (LI-COR, USA) which allows simultaneous measurements of chlorophyll fluorescence and gas exchange over the same leaf area (2 cm²). Measurements were conducted over two days in the summer, Feb 19 and Mar 4, in 2013 between 5:30 (predawn) and 19:30 (after-sunset) CAT. Two plants that had similar heights (1.2 m) and canopy architecture were chosen as representative. Three upper fully
expanded leaves on different branches of each plant were subjected to measurement. The leaves
were kept inside the chamber under irradiance from a blue and red LED source (10% blue) which
was tracking incident PAR measured outside the chamber by an external quantum sensor (model
LI-190SA, LI-COR) with a 3 second update rate. To avoid the chamber shading the external
quantum sensor, the sensor was placed on the top of the upper chamber and attached by double-
sided tape so that the sensor top was at the highest position during the measurement. No
environmental controls were performed; i.e., the block temperature was not controlled, and
incoming atmospheric air did not pass thorough either a desiccant or a CO₂ absorbing tube. A
semi-enclosed, empty 30-L water tank was used as a buffer volume to minimize instantaneous
fluctuations in incoming CO₂.

Minimal fluorescence ($F_0$) and maximal fluorescence in the darkness ($F_m$) were
measured at pre-dawn and 20–30 min after sunset and the maximal photochemical efficiency of
photosystem II (PSII) in darkness $[(F_m - F_o)/F_m = F_v/F_m]$ was calculated. Once steady-state
photosynthesis was achieved (generally within 3 min) standard gas exchange parameters along
with steady-state fluorescence ($F_s$) and maximal fluorescence in light ($F_m'$) were sampled at
various times during the day. On 4 Mar, it took additional minutes to wait for $F_s$ to be stable under
direct solar radiation through the clouds. The PSII quantum yield in the light $[\Phi_{PSII} : (F_m' - F_s)/F_m' =
1 - F_s/F_m']$ was calculated according to Genty et al. [23]. To quantify the partitioning of excitation
energy to the other processes, the quantum yield of non-photochemical quenching ($\Phi_{NPQ}$) and
either fluorescence or constitutive thermal dissipation ($\Phi_{f,D}$) were calculated according to
Hendrickson et al. [20] as follows:

\[
\Phi_{NPQ} = \frac{F_s}{F_m'} - \frac{F_s}{F_m} \tag{1}
\]
\[
\Phi_{f,D} = \frac{F_s}{F_m} \tag{2}
\]

Where $\Phi_{NPQ}$ represents xanthophyll and ΔpH-regulated heat dissipation, the major variable
components of heat dissipation [20], accounting for the proportion of quanta absorbed by PSII that
is used in these energy-dissipating processes, and thus represents the quantum efficiency in an
analogous way to $\Phi_{PSII}$ [20]. $\Phi_{f,D}$ is the combined quantum efficiency of fluorescence and
constitutive thermal dissipation [20]. \( F_m \) measured pre-dawn in each leaf was used for the

calculation of Eqn (1) and (2), assuming that the PSII photoinactivation rate, i.e., the degradation

of functional PSI reaction centers should be similar to the recovery rate since it is often difficult

to detect a net loss of PSII activity in nature [24]. The settings of light intensity for both the

saturating pulse and measuring pulse were constant at the measurement of both \( F_m \) and \( F_m' \).

Saturation pulses of about 8000 μmol m\(^{-2}\) s\(^{-1}\) were applied to achieve a complete saturation of the

PSII reaction centers. To minimize the effect of spatial heterogeneity in a leaf on absolute

fluorescence values, the measurement was performed at the same area of the leaf with care. In

addition, the chamber equipped with a chlorophyll fluorescence measurement system allowed the

measuring light source and fluorescence detector to keep the same distance with the leaves, thus

helping comparisons of the absolute fluorescence value in the same leaf. The rate of electron

transport utilized via photochemistry (\( J_{PSII} \)) was estimated after Krall and Edwards [25], by

multiplying \( \Phi_{PSII} \times \) incident PAR \( \times 0.5 \) (equal distribution of excitation of electrons per absorbed

photons between photosystems II and I is assumed), and \( \times 0.84 \) which is considered the most

common leaf absorbance coefficient for C3 plants [26]. Similarly, it is possible to estimate the rate

of energy dissipation via non-photochemical processes, \( J_{PSII} \) and \( J_{NPQ} \), by substituting \( \Phi_{PSII} \) for

\( \Phi_{NPQ} \) and \( \Phi_{f,D} \), respectively. Besides the measurement of PAR by the portable photosynthesis

system, PAR incident on the field at a horizontal plane was measured by another quantum sensor

(LI-190SA, LI-COR). The mA output of the quantum sensor was converted to mV through a

resister (2290 Millivolt Adapter, LI-COR) and taken by a portable datalogger (Datum-Y,

Yokogawa, Japan) every 10 sec.


2.3. Water treatment

A water recovery treatment was conducted on one of the two plants to assess the effects

of water stress on photosynthesis. The treatment started just after the measurement on Feb 19 with

a 5 L-water supply in a 0.6-m radius around the base of the main stem. The soil moisture was

measurement with a soil profile probe (Theta Probe type PR2/4, Delta-T devices) in the 0.3-m

radius. The change in soil moisture during the course of the treatment is shown in Figure 1.
Starting the next day, 5 L of water was supplied every day in the late afternoon (ca. 6 pm) for 4 days. From Feb 23, the water supply was further increased to 10 L because the soil moisture profile showed an increase in only the shallow soil layer (10 cm) in spite of the continuous treatment (Fig. 1). The 10-L water supply continued until 3 Mar, the day before the second measurement. The total amount of water supplied was 100 L per plant (equivalent to 88.5 mm of precipitation to the area watered). Between the two measurement days, the weather in the field was within typical summer conditions for the region: no major precipitation, high irradiance and air temperature usually reaching over 40°C on sunny days but lower on cloudy days.

2.5. Statistical analyses

Data obtained from 6 replicates on Feb 19 and 3 replicates with or without water treatment on Mar 4 were analyzed by a Tukey-Kramer test. Analysis of covariance (ANCOVA) was applied to analyze the effects of either the water treatment or the measurement days on $A–g_s$ relationships, $\Phi_{PSII}–\Phi_{NPQ}$ relationships, and light responses of $\Phi_{PSII}$. All statistical analyses were conducted using the R statistical software package [27]. Results were assumed to be significant at $P < 0.05$. 

Figure 1 here
3. Results

3.1. Diurnal change in the assimilation rate in relation to stomatal conductance

On Feb 19 (pretreatment), soil moisture down to 30 cm depth was under 9% and increased to 16, 11, and 10% at depths of 10, 20, and 30 cm, respectively, on May 4 with water treatment (Fig. 1). A nearly symmetric course of radiation was observed on Feb 19, while erratic radiation due to cloud cover was observed on May 4 (data not shown). Accordingly, the daily total PAR at the horizontal plane of 58.9 mol m\(^{-2}\) on Feb 19 was larger than on Mar 4 by 10.2 mol m\(^{-2}\).

The net CO\(_2\) assimilation rate (\(A\)) reached its maximum (\(A_{\text{max}}\)) in the range between 9.0 and 20.0 μmol m\(^{-2}\) s\(^{-1}\) in the early morning (ca. 7:00–9:00 am) of Feb 19 and declined to a negative value throughout the day (Fig. 2a). A similar pattern of depressed \(A\) was seen in the control, though the depression was less than in the pretreatment, i.e., the \(A\) never became negative (Fig. 2). The diurnal depression in \(A\) was mitigated under the water treatment Mar 4 (Fig. 2b). The \(A_{\text{max}}\) in the water treatment and control had ranges of 7.6–18.1 and 6.9–14.0 μmol m\(^{-2}\) s\(^{-1}\), respectively. In the water treatment, the \(A\) also declined after the \(A_{\text{max}}\) but slower than the quick drop seen in the control. The higher \(A\) was achieved at higher PAR values (>1000 mmol m\(^{-2}\) s\(^{-1}\)) in the water treatment while the control and pretreatment \(Jatropha\) leaves showed inhibited \(A\) in this range of irradiance. Consequently, the water treatment more than doubled the daily net CO\(_2\) assimilation (\(\Sigma\)) (Table 1). In comparison to the daily total PAR measured on a horizontal plane, the sum of the PAR incident on the leaves at the photosynthesis measurements (\(\Sigma_{\text{PAR}}\)) was apparently overestimated on Mar 4. This is because the \(\Sigma_{\text{PAR}}\) on Mar 4 was calculated mainly by summing the irradiance levels through the clouds measured for relatively longer intervals than the measurements on the horizontal plane.

On both measurement days, the stomatal conductance of water vapor (\(g_s\)) matched the changes observed over the period of \(A\) (Fig. 2ab). The maximal \(g_s\) (\(g_{\text{max}}\)) had a range of 70–230 mmol m\(^{-2}\) s\(^{-1}\) for the pretreatment, and 150–200 mmol m\(^{-2}\) s\(^{-1}\) for the water treatment and 80–130 mmol m\(^{-2}\) s\(^{-1}\) for the control. All of the \(g_{\text{max}}\) were observed in the early morning (ca. 8:00–9:00), corresponding to the time of \(A_{\text{max}}\). As a result, \(A\) was linearly correlated with \(g_s\) in the pretreatment.
The regression lines of the $A$–$g_s$ relationship were similar to each other, i.e., the slopes of the regression lines were 98, 113, and 102 μmol mmol$^{-1}$ and the $y$-intercepts were -1.5, -1.3, and -1.3 μmol m$^{-2}$ s$^{-1}$ in the pretreatment, control, and water treatment, respectively. According to an ANCOVA, there was no significant difference in the regression lines of the control and water treatment, suggesting no significant effect of water treatment on this relationship. The diurnal stomatal closure ($g_s < 50$ mmol m$^{-2}$ s$^{-1}$) prevented water loss through transpiration, holding $E$ within 2 mmol m$^{-2}$ s$^{-1}$ in either the pretreatment or the control (Fig. 2a). Clearly, the stomata in the water treatment stayed open, increasing $E$ as a result (Fig. 2b). With little transpirational cooling and high air temperatures due to the uninterrupted solar radiation, the leaf temperature increased to 45°C, leading to an extremely high leaf vapor pressure deficit (VPD) of 8 kPa on Feb 19 (Fig. 2a). In contrast, with the intermittent direct solar radiation on Mar 4 the leaf temperature increased to only 36°C and VPD to 4.5 kPa. On both measurement days, the $g_s$ sharply increased towards its maximum at a VPD of -2 kPa and dropped to its minimum (<40 mmol m$^{-2}$ s$^{-1}$) at around 4 kPa (Fig. 3). The $g_s$ also responded similarly to the VPAir regardless of the treatment (Fig. 3). Diurnally, the VPAir outside the chamber (measured for reference) fluctuated similarly to the one inside the chamber, suggesting that the VPAir inside the chamber was not elucidated by the transpiration via stomatal behavior (data not shown). The $g_s$ sensitively decreased at relatively high VPAir as VPAir decreased until $g_s$ reached its minimum. The $g_s$ relative to either VPAir or VPD in the water treatment tended to be higher than in the control.

3.2. Diurnal change in electron transport rate and photon partitioning in PSII

The diurnal course of the electron transport rate via photochemistry ($J_{PSII}$) followed the $A$ in the pretreatment and the control (Figs. 2ab and 4). Compared to the drop in $A$, the $J_{PSII}$ decreased moderately in the pretreatment and the control, while it stayed relatively high in the water treatment. In the pretreatment, the $J_{PSII}$ stayed stable with the value lower than 50 μmol m$^{-2}$ s$^{-1}$ in the afternoon (Fig. 4). The balance between the electron transport rate in PSII and $A$ ($J_{PSII}/A$) dynamically changed in each treatment (data not shown). The $J_{PSII}$ increased proportionally to $A$ up
to $A_{max}$, yielding a relatively constant $J_{PSII}/A$. Then, the $J_{PSII}/A$ increased because of the depression in $A$. There is no significant difference of the dark $F_{v}/F_{m}$ among treatment. The average $F_{v}/F_{m}$ was 0.83 ± 0.01 at pre-dawn on both Feb 19 and Mar 4, which slightly decreased to 0.78 ± 0.02 and 0.81 ± 0.02, respectively, after sunset (Fig. 4). The diurnal course of the quantum efficiency of photochemistry ($\Phi_{PSII}$) briefly mirrored the PAR (Figs. 2ab and 4). The mean minimum $\Phi_{PSII}$ reached down to 0.037 ± 0.003, 0.085 ± 0.025, and 0.14 ± 0.05 in the pretreatment, control, and water treatment, respectively. The quantum yield of the basal heat and fluorescence ($\Phi_{f,D}$) was a diurnally stable parameter with a value around 0.2 whereas the quantum efficiency of thermal dissipation ($\Phi_{NPQ}$) fluctuated in the opposite direction from the $\Phi_{PSII}$. A highly significant negative correlation between the $\Phi_{NPQ}$ and the $\Phi_{PSII}$ was seen with determination factors of 0.99 ($P < 0.001$) for each measurement day and treatment. The water treatment did not significantly alter this relationship. Furthermore, a great similarity was observed in this relationship between both measurement days, i.e., comparable slopes and intercepts with values of 0.895 and 0.755, respectively. The large fraction of the absorbed energy was utilized via regulated thermal dissipation when the $\Phi_{PSII}$ was extremely low (Fig. 4). The mean maximum $\Phi_{NPQ}$ achieved in the pretreatment, the control, and the water treatment were 0.80 ± 0.01, 0.76 ± 0.04, and 0.68 ± 0.04, respectively. In addition, either $\Phi_{PSII}$ or $\Phi_{NPQ}$ was sustained at a low level or high level, respectively, even while PAR was decreasing on Feb 19 (Fig. 5). However, both $\Phi_{PSII}$ and $\Phi_{NPQ}$ returned to their origin at dark (Figs. 4 and 5). The $\Phi_{PSII}$ started to increase at around 16:00 (Fig. 4). On 4 Mar, on the other hand, the sustained downregulation of $\Phi_{PSII}$ was not observed in either the control or the water treatment and an exponential curve was well fitted to the relationship, as shown in Figure 5. The slope of the log-transformed regression line became significantly less steep in the water treatment than in the control, suggesting that water treatment mitigated the PSII down regulation.

The strong dependence of excitation energy dissipation by NPQ was further highlighted in the estimation of the daily fraction of absorbed energy utilized via each pathway (Table 1). The total amount of absorbed energy utilized via regulated thermal dissipation ($\Sigma_{NPQ}$) was responsible for the largest fraction of the daily-absorbed energy up to about 72 %. Water treatment significantly decreased the fraction of $\Sigma_{NPQ}$ by 9%. In contrast, the fraction of daily absorbed light
utilized via constitutive thermal dissipation ($\Sigma_{f,I}$) was constant among the treatments with the value around 18%. Without water treatment, the fraction of daily-absorbed light utilized via photochemical pathways ($\Sigma_{PSII}$) was only 10.7 and 16.3% on Feb 19 and Mar 4, respectively. In the water treatment, the fraction of $\Sigma_{PSII}$ significantly increased by the same degree as the decrease in the fraction of $\Sigma_{NPQ}$. Since water treatment mitigated the drop in $A$ the daily total $J_{PSII}$ relative to $A$ ($\Sigma_{PSII}/\Sigma_A$) was the lowest for the water-treated Jatropha (Table 1). The highest $\Sigma_{PSII}/\Sigma_A$ was found with the lowest $\Sigma_A$ in the pretreatment, vice versa.
4. Discussion

4.1. Dynamic downregulation of diurnal photosynthesis under drought in Jatropha curcas

As was observed in this study, the asymmetrical patterns in $g_s$, as well as $A$ and $E$, with a predominant morning peak associated with severe water stress, have been reported in leaves for a wide variety of plant types and species [18]. In addition, a more than doubled daily CO₂ uptake after 13 d of water recovery treatment suggests that the Jatropha plants we measured had a shortage of water. On the other hand, the observed similarity in the $A$-$g_s$ linear relationships between the measurement days with and without water-treatment suggested that the diurnal stomatal closure was the predominant limitation of photosynthetic rates under water stress.

Stomata are known to sensitively respond to drought, which can be caused by either a dry atmosphere or dry soil, or both [28]. Midday stomatal closure is induced solely by simulated dry atmospheric conditions, suggesting that atmospheric conditions play the primary role [18]. Stomata close in direct response to dry air, which is triggered by the decreased turgor of peristomal cells, i.e., guard cells, sensitively responding to the increase in transpiration rate [28-30]. In this study, the diurnal course of leaf temperatures was the primary driver for diurnal changes in VPD, an indicator of the evapotranspirational demand (Fig. 2ab). Thus, the dynamic stomatal behavior after $g_{max}$ with increasing VPD can be interpreted as the effect of high leaf temperatures (Fig. 3). On the one hand, it is known that stomata respond less to the change in VPD triggered by leaf temperature than the change in VPD associated with $VP_{Air}$, possibly because of the positive feedback of the enhanced hydraulic conductivity to the evaporating site with increased leaf temperatures [31]. A positive relationship between $g_s$ and $VP_{Air}$ was also found on both measurement days (Fig. 3). Thus, we speculate that dynamic stomatal closure was the response to the atmospheric drought. Jatropha respond to decrease $g_s$ under progressive drought while maintaining the leaf water content [8,11], predawn leaf water potential [10], and the difference in leaf water potentials between predawn and midday as low as well watered plants [7], indicating that it has a desiccation postponement strategy [30]. The observed direct and quick stomatal response to the atmospheric drought would diurnally preserve the water status of the leaves, i.e.,
the maintenance of the leaves would otherwise be jeopardized by desiccative stress under limited
water availability. The conservative strategy of the diurnal stomatal closure would be related to the
slow uptake of water by shallow root system characterized by the poor absorbing roots with a
narrow and few xylem vessels in Jatropha [32]. Assumingly, the increase in the water uptake rate
with the increase in the root water availability might support the higher $g_s$ in the water treatment.

Compared to the diurnal stomatal regulation of $A$, the $J_{PSII}$ was not the limitation in $A$
since even the increase in the $J_{PSII}/A$ occurred during the depression in $A$ and that the
downregulation of $\Phi_{PSII}$ was highly dependent on the diurnal change in irradiance in a reversible
manner (Fig. 4). Similarly, the faster effect of the tight stomatal regulation compared to the
downregulation of $\Phi_{PSII}$ on the photosynthetic rate in Jatropha leaves was shown in a water stress-
recovery experiment with daily photosynthetic measurements [11]. The predawn $F_v/F_m$ of 0.83 on
each measurement day, the diurnal recovery of $\Phi_{PSII}$, and the marginal decrease in after sunset
$F_v/F_m$ without water treatment (Fig. 4) all indicate that the remarkably high stability of the PSII in
Jatropha under severe drought condition. The high PSII stability in Jatropha has also been found
under severe drought conditions that greatly suppressed $\Phi_{PSII}$ and $A$ [9,10]. While their findings
were based on pot experiments under relatively moderate controlled environments, our results
showed that Jatropha had high PSII stability under severe field conditions in a semi-arid. In
contrast, a large decline in $F_v/F_m$ (lower than 0.6) was observed in the leaves of Jatropha grown
under field conditions during the driest month in semi-arid habitat while $F_v/F_m$ was higher in the
other seasons [33].

4.2. Diurnal downregulation of PSII efficiency relating to thermal energy dissipation in Jatropha curcas

The daily integral of fluorescence and constitutive thermal dissipation ($\Sigma_{f,D}$) can be
contributed to a considerable fraction of the absorbed light energy under the drought. Indeed, it
exceeded the quantum yield of PSII on the daily basis (Table 1). The constant $\Phi_{f,D}$ and $\Sigma_{f,D}$
between the measurement days and treatment in our study strongly support the view that the
fluorescence and constitutive thermal dissipation is considered to be an inherent constitutive
thermal dissipation that occurs regardless of illumination conditions [20]. The downregulation of
$\Phi_{PSII}$ was proportional to the increase in $\Phi_{NPQ}$, indicating that *Jatropha* regulated $\Phi_{PSII}$ by
bypassing the absorbed energy via regulated thermal dissipation, namely zeathantin-facilitated
flexible thermal dissipation [16]. While the downregulation of $\Phi_{PSII}$ did not directly limit the
photosynthetic rate, a large fraction of energy absorbed was dissipated via the regulated thermal
dissipation when the energy demand for the CO$_2$ fixation was extremely low. The substantial
fraction of the $\Sigma_{NPQ}$ to the total absorbed energy for *Jatropha* without water treatment (66-72%) was the largest level among the ones confirmed in other species, i.e., 34% for sunflower, 51% for
Vinca major [19] and 54% for grapevine [20]. This is partly due to their growth condition with
lesser extent of stress, i.e., regular supply of water and fertilizer, since we found that the water
treatment decreased this fraction to 57% in *Jatropha*. In addition, the maximum value of $\Phi_{NPQ}$ in
*Jatropha* (80%) without water treatment is within the light saturating value ranging between 75
and 92% in the leaves of grapevines under extreme drought stress [34]. Likewise grapevine,
*Jatropha* leaves rely heavily upon thermal dissipation under drought condition.

We observed either sustained low levels of $\Phi_{PSII}$ or high levels of $\Phi_{NPQ}$ during the light
decreasing phase on Feb 19, which probably related to regulation of thermal dissipation via a
trans-thylakoid pH gradient ($\Delta$pH) associated with ATP utilization [35]. Regulated thermal
dissipation is flexibly facilitated by the lumen [H$^+$] and hence, $\Delta$pH. The absorption of sunlight
increased electron flow, resulting in a build-up of $\Delta$pH that is, in turn, dissipated by ATP
production via ATP synthase. Under the conditions representing light absorption that exceeds ATP
utilization, $\Delta$pH increases. The largely depressed $A$ with larger radiation loads on Feb 19 compared
to Mar 4 (Fig. 2ab) suggested that the relative rate of energy demand for the CO$_2$ fixation as a
fraction of the unavoidable energy supply was lower on Feb 19 than on Mar 4. Consequently, the
decrease in the rate of ATP consumption would result in a backlog of lumen protons, while the
increase in thylakoid membrane leakiness and $\Delta$pH gradient at high temperatures might be
stabilized via feedback from zeaxanthin [36] and cyclic phosphorylation [37,38], respectively. In
another possibility, there might be misassumption in the model we applied that there was no
photoinactivation of PSII, i.e., the quantum efficiency of thermal dissipation by inactive PSII. The
fraction of functional PSII is dependent on the rate of repair via D1 synthesis relative to
photoinactivation with respect to photon exposure and irradiance [24]. Under environmental conditions that are unfavorable for the photosynthetic fixation of CO₂, an excess of light energy absorbed by photosynthetic pigments suppresses the translation of factors required for D1 synthesis and, thus, increases the extent of photoactivated PSII, namely photodamage [39]. In such cases, the Φ_{NPQ} is overestimated with an increase in the fraction of photoinactivated PSII [21]. If the sustained Φ_{NPQ} is elucidated by the inactivated PSII, the overestimation of Φ_{f,D} also occurs during the time of sustained Φ_{NPQ} (Fig. 4; 13:00–19:00). However, an increase in Φ_{f,D} wasn’t apparent, indicating that this possibility is unlikely. Although the process underlying the sustained high levels of thermal dissipation was not clear in this study, J_{PSII} remained stable instead of increasing further (Fig. 4). In addition to this, the lowest Σ_{PSII} corresponded to the lowest Σ_{f} on Feb 19 (Table 1) suggests that the diurnal downregulation of Φ_{PSII} via the sustained thermal dissipation is an additional photoprotective function.

The diurnal increase in J_{PSII}/A after the depression in A and the higher Σ_{PSII}/Σ_{f} without water treatment indicate an increase in the proportion of photorespiration [9,15] and/or alternative photochemical rates, such as the water-water cycle [40] under the inhibited A. In either process, reactive oxygen species, i.e., O₂⁻ and H₂O₂, which potentially cause oxidative stress including degradation of photosynthetic pigments, are inevitably produced [40]. It is known that the activities of antioxidants including superoxide dismutase, catalase and glutamine synthetase significantly increased in the water-stressed Jatropha [8]. In addition, the large increase in the activity of ascorbate peroxidase, the core H₂O₂ scavenging enzyme of higher plants, coordinated with the increase in J_{PSII}/A ratio as the water deficit increased was found in Jatropha [9]. Accordingly, the Jatropha plants could have had a high antioxidant enzyme activity in the acclimation of the drought environment, which might also have contributed to preserving the integrity of the PSII.
5. Conclusions

Our field study strongly supports the previous findings that the main drought resistance strategy of *Jatropha* is via strict stomatal regulation. The field-grown *Jatropha* plants under water deficit showed sensitive stomatal regulation on photosynthesis, being coordinated with a drying atmosphere in a semi-arid habitat. Even though the risk of excess excitation energy which potentially causes photodamage increased with the extremely low capacity for CO₂ fixation, *Jatropha* preserved the integrity of PSII. This was safely accomplished by the regulated thermal energy dissipation mediated by the xanthophyll cycle, which accounted a large fraction of both instantaneous and daily absorbed energy. In addition, sustained downregulation of PSII quantum efficiency via the regulated thermal dissipation was observed and thought to be an additional photoprotective function. Results of the water treatment showed that *Jatropha* has high capacity for regulated thermal energy dissipation that flexibly adjust PSII quantum efficiency to the capacity of CO₂ fixation and must be critically important for this strict water conserving species, especially under a climate with high solar radiation loads as was seen in this semi-arid region.
Acknowledgements

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References


Captions

Table 1. Mean total daily integrated irradiance incident on the leaves (\(\Sigma_{PAR}\)), net CO\(_2\) assimilation rate (\(\Sigma_i\)), the total amount of absorbed energy utilized via PSII photochemistry (\(\Sigma_{PSII}\)) and lost via regulated thermal dissipation (\(\Sigma_{NPQ}\)) and the sum of fluorescence quenching and constitutive thermal dissipation (\(\Sigma_{f,D}\)), and the ratio \(\Sigma_{PSII}/\Sigma_i\) for field grown \textit{Jatropha} leaves (\textit{Jatropha curcas}) on each measurement day. The fraction of the total absorbed radiation passing through Photosystem II that is consumed via each of the three processes is presented in brackets.

Figure 1. Change in volumetric soil water content at various soil depths (10, 20, and 30 cm) days after the first water treatment (DAW). Water was supplied every late afternoon in the amount of 5 L until 4 DAW and 10 L until 13 DAW.

Figure 2a. Diurnal time courses of photosynthetic active radiation (PAR), net CO\(_2\) assimilation rate (\(A\)), stomatal conductance (\(g_s\)), leaf temperature, leaf and air vapor pressure, and transpiration rate (\(E\)) on Feb 19 (pretreatment). Different symbols represent different trees. Values are means ± SE of 3 values.

Figure 2b. Diurnal time courses of photosynthetic active radiation (PAR), net CO\(_2\) assimilation rate (\(A\)), stomatal conductance (\(g_s\)), leaf temperature, leaf and air vapor pressure, and transpiration rate (\(E\)) on May 4 (control and water treatment). For different symbols, see Figure 2a. Black-filled circles represent water treatment. Values are means ± SE of 3 values.

Figure 3. Relationship between stomatal conductance (\(g_s\)) and vapor pressure difference (VPD) and atmospheric vapor pressure (VP\(_{Air}\)) on Feb 19 (pretreatment) and on Mar 4 (control and water treatment). Values are single measurements from 6 equivalent leaves on each measurement day, taken during the full daily cycle. Black-filled circles represent water treatment, while gray-filled and open circles represent pretreatment and control, respectively.
Figure 4. Diurnal time courses of rate of electron transport utilized via photochemistry ($J_{PSII}$), quantum efficiency of photochemical transport in PSII ($\Phi_{PSII}$ and $F_m/F_s$ in the dark), quantum yield of basal heat and fluorescence ($\Phi_f$) and quantum efficiency of thermal dissipation ($\Phi_{NPQ}$) on Feb 19 (pretreatment; left) and Mar 4 (control and water treatment; right). For the different symbols, see Figure 2ab. Values are means ± SE of 3 values.

Figure 5. Relationship between quantum efficiency of photochemical transport in PSII ($\Phi_{PSII}$) and quantum efficiency of thermal dissipation ($\Phi_{NPQ}$) and photosynthetic active radiation (PAR) on Feb 19 (pretreatment; left) and Mar 4 (control and water treatment; right). Values are single measurements from 6 equivalent leaves on each measurement day, taken during the full daily cycle. On Mar 4 (right graph), black-filled circles represent water treatment.
### Table 1

<table>
<thead>
<tr>
<th>Date</th>
<th>( \Sigma_{PAR} )</th>
<th>( \Sigma_A )</th>
<th>( \Sigma_{f,D} )</th>
<th>( \Sigma_{NPQ} )</th>
<th>( \Sigma_{PSII} )</th>
<th>( \Sigma_{PSII} )/( \Sigma_A )</th>
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<tr>
<td>Feb 19 (pretreatment)</td>
<td>57.3(^a)</td>
<td>72.4(^b)</td>
<td>4.3(^a)</td>
<td>17.4(^a)</td>
<td>2.6(^c)</td>
<td>37.1(^a)</td>
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<tr>
<td>(control)</td>
<td>55.9(^a)</td>
<td>139.6(^b)</td>
<td>4.4(^a)</td>
<td>16.1(^{ab})</td>
<td>4.4(^b)</td>
<td>29.1(^{ab})</td>
</tr>
<tr>
<td>Mar 4 (water treatment)</td>
<td>52.5(^a)</td>
<td>330.9(^a)</td>
<td>4.2(^a)</td>
<td>13.1(^b)</td>
<td>5.7(^a)</td>
<td>18.0(^b)</td>
</tr>
</tbody>
</table>

*Tukey-Kramer, \( P < 0.05 \)
Fig 2a

[Graph showing the relationship between PAR (μmol m⁻² s⁻¹), A (μmol m⁻² s⁻¹), gₛ (mmol m⁻² s⁻¹), and E (mmol m⁻² s⁻¹) over time (CAT, h)]

- PAR (μmol m⁻² s⁻¹)
- A (μmol m⁻² s⁻¹)
- gₛ (mmol m⁻² s⁻¹)
- E (mmol m⁻² s⁻¹)

Leaf temperature (°C) and VP (kPa) are also plotted on the right side of the graph.

Time (CAT, h) from 5 to 19 is shown on the x-axis and the y-axes represent the respective variables.
Fig 2b

![Graph showing PAR (µmol m² s⁻¹), A (µmol m² s⁻¹), gₛ (mmol m⁻² s⁻¹), Leaf temperature (°C), and VP (kPa) over time (CAT, h).]
Fig 3

A graph showing the relationship between stomatal conductance ($g_s$) in mmol m$^{-2}$ s$^{-1}$ and VPD (kPa) for different treatments. The graph includes data points for pretreatment, control, and water treatment conditions. The inset graph focuses on a specific range of VPD values. The x-axis represents VPD (kPa) ranging from 0 to 10, and the y-axis represents $g_s$ (mmol m$^{-2}$ s$^{-1}$) ranging from 0 to 300.
Fig 5

$\Phi_{\text{PSII}}$, $\Phi_{\text{NPQ}}$

PAR (µmol m\(^{-2}\) s\(^{-1}\))

$y = 0.767 \exp^{-0.71x}$
$R^2 = 0.936^{***}$

$y = 0.750 \exp^{-0.94x}$
$R^2 = 0.910^{***}$