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

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21 **Abstract**

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

40

41 **Key words:** Drought stress, Diurnal photosynthesis, Quenching partitioning, Non-photochemical
42 quenching, Thermal dissipation, Semi-arid

43 **1. Introduction**

44

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

53 Photosynthesis is the primary source of plant production and is also among the primary
54 processes to be affected by drought [5,6]. Recently, increasing number of studies investigate
55 photosynthetic response to drought in *Jatropha*. Strict stomatal regulation in *Jatropha* under water
56 deficit has been reported in previous studies [7-11]. Acclimation of osmotic adjustment by means
57 of increases in inorganic [10] and organic solutes [12] and of increased activities of several
58 antioxidant enzymes [8,9] to prolonged water stress coordinated with a decrease in photosynthetic
59 rate (A) have also been observed. Some studies applied simultaneous measurements of chlorophyll
60 fluorescence and gas-exchange rates to *Jatropha* leaves. The progressive breakage of the link
61 between the electron transport rate and CO_2 assimilation rate were found under drought in
62 *Jatropha* [9], indicating that the alternative electron flow mediates the antioxidant activity in
63 water-stressed *Jatropha* leaves. Chronic photoinhibition, indicated by decreases in predawn F_v/F_m
64 [13], is rarely seen under moderate to severe drought even when the photosynthetic rate is strongly
65 impaired [9,10,14,15]. Instead, the increase in non-photochemical quenching (NPQ), namely
66 rapidly reversible components of thermal dissipation [16], under the water deficit as well as
67 downregulation of PSII quantum efficiency (Φ_{PSII}) were reported and thought to have
68 photoprotective functions via dissipation of excess energy at the PSII [9,15]. These different levels
69 of characteristic responses to water stress in *Jatropha* however, have been identified under
70 controlled, relatively mild climatic conditions with pot experiments, i.e., relatively low light
71 intensity and moderate temperature and humidity. In semi-arid climates, such as in the

72 Mediterranean region, where a scarcity of precipitation generally coincides with sunny days, water
73 stress on photosynthesis might be further exacerbated by the excess solar radiation loads and other
74 co-factors, such as high temperatures and evaporative water demand [17]. In addition, plants in
75 arid habitats are known to conserve water by means of midday stomatal closure [18], which is
76 often either excluded or discounted in a controlled experiment. Accordingly, it is difficult to assess
77 the relative importance of these photosynthetic responses to drought without understanding the
78 field performance under drought condition. However, a few studies have assessed diurnal
79 photosynthesis in *Jatropha* grown in semi-arid where plants were exposed to water deficits with
80 multiple stress factors.

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

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

94

95 **2. Materials and methods**

96

97 *2.1. Experimental site*

98

99 The experimental site was an agricultural field located in the Department of Agricultural
100 Research, Ministry of Agriculture of Botswana (25°56'37"E, 24°33'40"S, 992 m a.s.l.). The area is
101 categorized as a semi-arid climate [22], with a wide range of diurnal temperatures throughout the
102 year. In the summer season, the lowest temperature reaches below 15°C in the morning whereas
103 the highest temperature reaches over 40°C at midday. Mean annual precipitation is less than 490
104 mm on average, which is lower than what is considered the optimal value of between 1000 and
105 1500 mm for the growth of *Jatropha* [4]. The precipitation in 2012 at the experimental site was
106 only 333 mm. Rainfall mainly occurs during October and March, accounting for almost 100% of
107 the annual rainfall. The reddish-brown soil is categorized as a Rendzic Leptosol, with poor fertility,
108 high aluminum and iron, and mainly consisting of silt and clay (personal communication). Healthy
109 *Jatropha* seedlings from the seeds of Botswana accessions were transplanted into the 0.5 ha field
110 with a spacing of 2 m × 2 m in Dec 2011. Manual irrigation was performed from Dec 2011 until
111 July 2012, and a drip irrigation system was installed in July 2012 that provided 5 L week⁻¹ of
112 water per plant during the rest of the dry season until Oct 2012. From Nov 2012, the irrigation
113 stopped and the only water supply was from rain. A pruning treatment was done in May 2012 for
114 overcoming winter coldness, and 90% of the *Jatropha* plants survived through the winter in 2012.

115

116 *2.2. Diurnal gas exchange and chlorophyll fluorescence*

117

118 In situ gas-exchange and fluorescence parameters were measured in *Jatropha* with a
119 portable photosynthesis system equipped with a 6400-40 leaf chamber fluorometer (LI-COR,
120 USA) which allows simultaneous measurements of chlorophyll fluorescence and gas exchange
121 over the same leaf area (2 cm²). Measurements were conducted over two days in the summer, Feb
122 19 and Mar 4, in 2013 between 5:30 (predawn) and 19:30 (after-sunset) CAT. Two plants that had
123 similar heights (1.2 m) and canopy architecture were chosen as representative. Three upper fully

124 expanded leaves on different branches of each plant were subjected to measurement. The leaves
 125 were kept inside the chamber under irradiance from a blue and red LED source (10% blue) which
 126 was tracking incident PAR measured outside the chamber by an external quantum sensor (model
 127 LI-190SA, LI-COR) with a 3 second update rate. To avoid the chamber shading the external
 128 quantum sensor, the sensor was placed on the top of the upper chamber and attached by double-
 129 sided tape so that the sensor top was at the highest position during the measurement. No
 130 environmental controls were performed; i.e., the block temperature was not controlled, and
 131 incoming atmospheric air did not pass thorough either a desiccant or a CO₂ absorbing tube. A
 132 semi-enclosed, empty 30-L water tank was used as a buffer volume to minimize instantaneous
 133 fluctuations in incoming CO₂.

134 Minimal fluorescence (F_o) and maximal fluorescence in the darkness (F_m) were
 135 measured at pre-dawn and 20–30 min after sunset and the maximal photochemical efficiency of
 136 photosystem II (PSII) in darkness [$(F_m - F_o)/F_m = F_v/F_m$] was calculated. Once steady-state
 137 photosynthesis was achieved (generally within 3 min) standard gas exchange parameters along
 138 with steady-state fluorescence (F_s) and maximal fluorescence in light (F_m') were sampled at
 139 various times during the day. On 4 Mar, it took additional minutes to wait for F_s to be stable under
 140 direct solar radiation through the clouds. The PSII quantum yield in the light [$\Phi_{PSII}: (F_m' - F_s)/F_m =$
 141 $1 - F_s/F_m'$] was calculated according to Genty et al. [23]. To quantify the partitioning of excitation
 142 energy to the other processes, the quantum yield of non-photochemical quenching (Φ_{NPQ}) and
 143 either fluorescence or constitutive thermal dissipation ($\Phi_{f,D}$) were calculated according to
 144 Hendrickson et al. [20] as follows:

145

$$146 \quad \Phi_{NPQ} = F_s/F_m' - F_s/F_m \quad (1)$$

$$147 \quad \Phi_{f,D} = F_s/F_m \quad (2)$$

148

149 Where Φ_{NPQ} represents xanthophyll and ΔpH -regulated heat dissipation, the major variable
 150 components of heat dissipation [20], accounting for the proportion of quanta absorbed by PSII that
 151 is used in these energy-dissipating processes, and thus represents the quantum efficiency in an
 152 analogous way to Φ_{PSII} [20]. $\Phi_{f,D}$ is the combined quantum efficiency of fluorescence and

153 constitutive thermal dissipation [20]. F_m measured pre-dawn in each leaf was used for the
 154 calculation of Eqn (1) and (2), assuming that the PSII photoinactivation rate, i.e., the degradation
 155 of functional PSII reaction centers should be similar to the recovery rate since it is often difficult
 156 to detect a net loss of PSII activity in nature [24]. The settings of light intensity for both the
 157 saturating pulse and measuring pulse were constant at the measurement of both F_m and F_m' .
 158 Saturation pulses of about $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ were applied to achieve a complete saturation of the
 159 PSII reaction centers. To minimize the effect of spatial heterogeneity in a leaf on absolute
 160 fluorescence values, the measurement was performed at the same area of the leaf with care. In
 161 addition, the chamber equipped with a chlorophyll fluorescence measurement system allowed the
 162 measuring light source and fluorescence detector to keep the same distance with the leaves, thus
 163 helping comparisons of the absolute fluorescence value in the same leaf. The rate of electron
 164 transport utilized via photochemistry (J_{PSII}) was estimated after Krall and Edwards [25], by
 165 multiplying $\Phi_{PSII} \times \text{incident PAR} \times 0.5$ (equal distribution of excitation of electrons per absorbed
 166 photons between photosystems II and I is assumed), and $\times 0.84$ which is considered the most
 167 common leaf absorbance coefficient for C3 plants [26]. Similarly, it is possible to estimate the rate
 168 of energy dissipation via non-photochemical processes, J_{PSII} and J_{NPQ} , by substituting Φ_{PSII} for
 169 Φ_{NPQ} and $\Phi_{f,D}$, respectively. Besides the measurement of PAR by the portable photosynthesis
 170 system, PAR incident on the field at a horizontal plane was measured by another quantum sensor
 171 (LI-190SA, LI-COR). The mA output of the quantum sensor was converted to mV through a
 172 resistor (2290 Millivolt Adapter, LI-COR) and taken by a portable datalogger (Datum-Y,
 173 Yokogawa, Japan) every 10 sec.

174

175 2.3. Water treatment

176

177 A water recovery treatment was conducted on one of the two plants to assess the effects
 178 of water stress on photosynthesis. The treatment started just after the measurement on Feb 19 with
 179 a 5 L-water supply in a 0.6-m radius around the base of the main stem. The soil moisture was
 180 measurement with a soil profile probe (Theta Probe type PR2/4, Delta-T devices) in the 0.3-m
 181 radius. The change in soil moisture during the course of the treatment is shown in Figure 1.

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

190 Figure 1 here

191 2.5. Statistical analyses

192

193 Data obtained from 6 replicates on Feb 19 and 3 replicates with or without water
194 treatment on Mar 4 were analyzed by a Tukey-Kramer test. Analysis of covariance (ANCOVA)
195 was applied to analyze the effects of either the water treatment or the measurement days on $A-g_s$
196 relationships, Φ_{PSII} – Φ_{NPQ} relationships, and light responses of Φ_{PSII} . All statistical analyses were
197 conducted using the R statistical software package [27]. Results were assumed to be significant at
198 $P < 0.05$.

199

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 CO₂ assimilation rate (*A*) reached its maximum (*A_{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_{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_{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 CO₂ assimilation (Σ_{*A*}) (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 (Σ_{*P_{AR}*}) was apparently overestimated on Mar 4. This is because the Σ_{*P_{AR}*} 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_{smax}*) 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_{smax}* were observed in the early morning (ca. 8:00–9:00), corresponding to the time of *A_{max}*. As a result, *A* was linearly correlated with *g_s* in the pretreatment

229 ($R^2 = 0.834$, $P < 0.001$) and in the water treatment ($R^2 = 0.856$, $P < 0.001$) or the control ($R^2 =$
 230 0.903 , $P < 0.001$). The regression lines of the A - g_s relationship were similar to each other, i.e., the
 231 slopes of the regression lines were 98, 113, and 102 $\mu\text{mol mmol}^{-1}$ and the y-intercepts were -1.5, -
 232 1.3, and -1.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the pretreatment, control, and water treatment, respectively. According
 233 to an ANCOVA, there was no significant difference in the regression lines of the control and water
 234 treatment, suggesting no significant effect of water treatment on this relationship. The diurnal
 235 stomatal closure ($g_s < 50 \text{ mmol m}^{-2} \text{s}^{-1}$) prevented water loss through transpiration, holding E
 236 within 2 $\text{mmol m}^{-2} \text{s}^{-1}$ in either the pretreatment or the control (Fig. 2ab). Clearly, the stomata in
 237 the water treatment stayed open, increasing E as a result (Fig. 2b). With little transpirational
 238 cooling and high air temperatures due to the uninterrupted solar radiation, the leaf temperature
 239 increased to 45°C, leading to an extremely high leaf vapor pressure deficit (VPD) of 8 kPa on Feb
 240 19 (Fig. 2a). In contrast, with the intermittent direct solar radiation on Mar 4 the leaf temperature
 241 increased to only 36°C and VPD to 4.5 kPa. On both measurement days, the g_s sharply increased
 242 towards its maximum at a VPD of -2 kPa and dropped to its minimum ($<40 \text{ mmol m}^{-2} \text{s}^{-1}$) at
 243 around 4 kPa (Fig. 3). The g_s also responded similarly to the VP_{Air} regardless of the treatment
 244 (Fig. 3). Diurnally, the VP_{Air} outside the chamber (measured for reference) fluctuated similarly to
 245 the one inside the chamber, suggesting that the VP_{Air} inside the chamber was not elucidated by the
 246 transpiration via stomatal behavior (data not shown). The g_s sensitively decreased at relatively
 247 high VP_{Air} as VP_{Air} decreased until g_s reached its minimum. The g_s relative to either VP_{Air} or VPD
 248 in the water treatment tended to be higher than in the control.

249

250 3.2. Diurnal change in electron transport rate and photon partitioning in PSII

251

252 The diurnal course of the electron transport rate via photochemistry (J_{PSII}) followed the
 253 A in the pretreatment and the control (Figs. 2ab and 4). Compared to the drop in A , the J_{PSII}
 254 decreased moderately in the pretreatment and the control, while it stayed relatively high in the
 255 water treatment. In the pretreatment, the J_{PSII} stayed stable with the value lower than 50 $\mu\text{mol m}^{-2}$
 256 s^{-1} in the afternoon (Fig. 4). The balance between the electron transport rate in PSII and A (J_{PSII}/A)
 257 dynamically changed in each treatment (data not shown). The J_{PSII} increased proportionally to A up

258 to A_{max} , yielding a relatively constant J_{PSII}/A . Then, the J_{PSII}/A increased because of the depression
 259 in A . There is no significant difference of the dark F_v/F_m among treatment. The average F_v/F_m was
 260 0.83 ± 0.01 at pre-dawn on both Feb 19 and Mar 4, which slightly decreased to 0.78 ± 0.02 and
 261 0.81 ± 0.02 , respectively, after sunset (Fig. 4). The diurnal course of the quantum efficiency of
 262 photochemistry (Φ_{PSII}) briefly mirrored the PAR (Figs. 2ab and 4). The mean minimum Φ_{PSII}
 263 reached down to 0.037 ± 0.003 , 0.085 ± 0.025 , and 0.14 ± 0.05 in the pretreatment, control, and
 264 water treatment, respectively. The quantum yield of the basal heat and fluorescence ($\Phi_{f,D}$) was a
 265 diurnally stable parameter with a value around 0.2 whereas the quantum efficiency of thermal
 266 dissipation (Φ_{NPQ}) fluctuated in the opposite direction from the Φ_{PSII} . A highly significant negative
 267 correlation between the Φ_{NPQ} and the Φ_{PSII} was seen with determination factors of 0.99 ($P < 0.001$)
 268 for each measurement day and treatment. The water treatment did not significantly alter this
 269 relationship. Furthermore, a great similarity was observed in this relationship between both
 270 measurement days, i.e., comparable slopes and intercepts with values of 0.895 and 0.755,
 271 respectively. The large fraction of the absorbed energy was utilized via regulated thermal
 272 dissipation when the Φ_{PSII} was extremely low (Fig. 4). The mean maximum Φ_{NPQ} achieved in the
 273 pretreatment, the control, and the water treatment were 0.80 ± 0.01 , 0.76 ± 0.04 , and 0.68 ± 0.04 ,
 274 respectively. In addition, either Φ_{PSII} or Φ_{NPQ} was sustained at a low level or high level,
 275 respectively, even while PAR was decreasing on Feb 19 (Fig. 5). However, both Φ_{PSII} and Φ_{NPQ}
 276 returned to their origin at dark (Figs. 4 and 5). The Φ_{PSII} started to increase at around 16:00 (Fig.
 277 4). On 4 Mar, on the other hand, the sustained downregulation of Φ_{PSII} was not observed in either
 278 the control or the water treatment and an exponential curve was well fitted to the relationship, as
 279 shown in Figure 5. The slope of the log-transformed regression line became significantly less steep
 280 in the water treatment than in the control, suggesting that water treatment mitigated the PSII down
 281 regulation.

282 The strong dependence of excitation energy dissipation by NPQ was further highlighted
 283 in the estimation of the daily fraction of absorbed energy utilized via each pathway (Table 1). The
 284 total amount of absorbed energy utilized via regulated thermal dissipation (Σ_{NPQ}) was responsible
 285 for the largest fraction of the daily-absorbed energy up to about 72 %. Water treatment
 286 significantly decreased the fraction of Σ_{NPQ} by 9%. In contrast, the fraction of daily absorbed light

287 utilized via constitutive thermal dissipation (Σ_{fD}) was constant among the treatments with the
288 value around 18 %. Without water treatment, the fraction of daily-absorbed light utilized via
289 photochemical pathways (Σ_{PSII}) was only 10.7 and 16.3 % on Feb 19 and Mar 4, respectively. In
290 the water treatment, the fraction of Σ_{PSII} significantly increased by the same degree as the decrease
291 in the fraction of Σ_{NPQ} . Since water treatment mitigated the drop in A the daily total J_{PSII} relative to
292 A (Σ_{PSII} / Σ_A) was the lowest for the water-treated *Jatropha* (Table 1). The highest Σ_{PSII} / Σ_A was
293 found with the lowest Σ_A in the pretreatment, vice versa.
294

295 4. Discussion

296

297 4.1. Dynamic downregulation of diurnal photosynthesis under drought in *Jatropha curcas*

298

299 As was observed in this study, the asymmetrical patterns in g_s , as well as A and E , with a
300 predominant morning peak associated with severe water stress, have been reported in leaves for a
301 wide variety of plant types and species [18]. In addition, a more than doubled daily CO_2 uptake
302 after 13 d of water recovery treatment suggests that the *Jatropha* plants we measured had a
303 shortage of water. On the other hand, the observed similarity in the A – g_s linear relationships
304 between the measurement days with and without water-treatment suggested that the diurnal
305 stomatal closure was the predominant limitation of photosynthetic rates under water stress.

306 Stomata are known to sensitively respond to drought, which can be caused by either a
307 dry atmosphere or dry soil, or both [28]. Midday stomatal closure is induced solely by simulated
308 dry atmospheric conditions, suggesting that atmospheric conditions play the primary role [18].
309 Stomata close in direct response to dry air, which is triggered by the decreased turgor of
310 peristomatal cells, i.e., guard cells, sensitively responding to the increase in transpiration rate [28-
311 30]. In this study, the diurnal course of leaf temperatures was the primary driver for diurnal
312 changes in VPD, an indicator of the evapotranspirational demand (Fig. 2ab). Thus, the dynamic
313 stomatal behavior after g_{max} with increasing VPD can be interpreted as the effect of high leaf
314 temperatures (Fig. 3). On the one hand, it is known that stomata respond less to the change in VPD
315 triggered by leaf temperature than the change in VPD associated with VP_{Air} , possibly because of
316 the positive feedback of the enhanced hydraulic conductivity to the evaporating site with increased
317 leaf temperatures [31]. A positive relationship between g_s and VP_{Air} was also found on both
318 measurement days (Fig. 3). Thus, we speculate that dynamic stomatal closure was the response to
319 the atmospheric drought. *Jatropha* respond to decrease g_s under progressive drought while
320 maintaining the leaf water content [8,11], predawn leaf water potential [10], and the difference in
321 leaf water potentials between predawn and midday as low as well watered plants [7], indicating
322 that it has a desiccation postponement strategy [30]. The observed direct and quick stomatal
323 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 Φ_{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 Φ_{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 Φ_{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 Φ_{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

353 thermal dissipation that occurs regardless of illumination conditions [20]. The downregulation of
 354 Φ_{PSII} was proportional to the increase in Φ_{NPQ} , indicating that *Jatropha* regulated Φ_{PSII} by
 355 bypassing the absorbed energy via regulated thermal dissipation, namely zeaxanthin-facilitated
 356 flexible thermal dissipation [16]. While the downregulation of Φ_{PSII} did not directly limit the
 357 photosynthetic rate, a large fraction of energy absorbed was dissipated via the regulated thermal
 358 dissipation when the energy demand for the CO₂ fixation was extremely low. The substantial
 359 fraction of the Σ_{NPQ} to the total absorbed energy for *Jatropha* without water treatment (66-72%)
 360 was the largest level among the ones confirmed in other species, i.e., 34% for sunflower, 51% for
 361 *Vinca major* [19] and 54% for grapevine [20]. This is partly due to their growth condition with
 362 lesser extent of stress, i.e., regular supply of water and fertilizer, since we found that the water
 363 treatment decreased this fraction to 57% in *Jatropha*. In addition, the maximum value of Φ_{NPQ} in
 364 *Jatropha* (80%) without water treatment is within the light saturating value ranging between 75
 365 and 92% in the leaves of grapevines under extreme drought stress [34]. Likewise grapevine,
 366 *Jatropha* leaves rely heavily upon thermal dissipation under drought condition.

367 We observed either sustained low levels of Φ_{PSII} or high levels of Φ_{NPQ} during the light
 368 decreasing phase on Feb 19, which probably related to regulation of thermal dissipation via a
 369 trans-thylakoid pH gradient (ΔpH) associated with ATP utilization [35]. Regulated thermal
 370 dissipation is flexibly facilitated by the lumen [H^+] and hence, ΔpH . The absorption of sunlight
 371 increased electron flow, resulting in a build-up of ΔpH that is, in turn, dissipated by ATP
 372 production via ATP synthase. Under the conditions representing light absorption that exceeds ATP
 373 utilization, ΔpH increases. The largely depressed *A* with larger radiation loads on Feb 19 compared
 374 to Mar 4 (Fig. 2ab) suggested that the relative rate of energy demand for the CO₂ fixation as a
 375 fraction of the unavoidable energy supply was lower on Feb 19 than on Mar 4. Consequently, the
 376 decrease in the rate of ATP consumption would result in a backlog of lumen protons, while the
 377 increase in thylakoid membrane leakiness and ΔpH gradient at high temperatures might be
 378 stabilized via feedback from zeaxanthin [36] and cyclic phosphorylation [37,38], respectively. In
 379 another possibility, there might be misassumption in the model we applied that there was no
 380 photoinactivation of PSII, i.e., the quantum efficiency of thermal dissipation by inactive PSII. The
 381 fraction of functional PSII is dependent on the rate of repair via D1 synthesis relative to

382 photoinactivation with respect to photon exposure and irradiance [24]. Under environmental
 383 conditions that are unfavorable for the photosynthetic fixation of CO₂, an excess of light energy
 384 absorbed by photosynthetic pigments suppresses the translation of factors required for D1 synthesis
 385 and, thus, increases the extent of photoinactivated PSII, namely photodamage [39]. In such cases,
 386 the Φ_{NPQ} is overestimated with an increase in the fraction of photoinactivated PSII [21]. If the
 387 sustained Φ_{NPQ} is elucidated by the inactivated PSII, the overestimation of Φ_{fD} also occurs during
 388 the time of sustained Φ_{NPQ} (Fig. 4; 13:00–19:00). However, an increase in Φ_{fD} wasn't apparent,
 389 indicating that this possibility is unlikely. Although the process underlying the sustained high
 390 levels of thermal dissipation was not clear in this study, J_{PSII} remained stable instead of increasing
 391 further (Fig. 4). In addition to this, the lowest Σ_{PSII} corresponded to the lowest Σ_A on Feb 19 (Table
 392 1) suggests that the diurnal downregulation of Φ_{PSII} via the sustained thermal dissipation is an
 393 additional photoprotective function.

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

406

407 **5. Conclusions**

408 Our field study strongly supports the previous findings that the main drought resistance
409 strategy of *Jatropha* is via strict stomatal regulation. The field-grown *Jatropha* plants under water
410 deficit showed sensitive stomatal regulation on photosynthesis, being coordinated with a drying
411 atmosphere in a semi-arid habitat. Even though the risk of excess excitation energy which
412 potentially causes photodamage increased with the extremely low capacity for CO₂ fixation,
413 *Jatropha* preserved the integrity of PSII. This was safely accomplished by the regulated thermal
414 energy dissipation mediated by the xanthophyll cycle, which accounted a large fraction of both
415 instantaneous and daily absorbed energy. In addition, sustained downregulation of PSII quantum
416 efficiency via the regulated thermal dissipation was observed and thought to be an additional
417 photoprotective function. Results of the water treatment showed that *Jatropha* has high capacity
418 for regulated thermal energy dissipation that flexibly adjust PSII quantum efficiency to the
419 capacity of CO₂ fixation and must be critically important for this strict water conserving species,
420 especially under a climate with high solar radiation loads as was seen in this semi-arid region.
421

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423

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Captions

Table 1. Mean total daily integrated irradiance incident on the leaves (Σ_{PAR}), net CO₂ assimilation rate (Σ_A), the total amount of absorbed energy utilized via PSII photochemistry (Σ_{PSII}) and lost via regulated thermal dissipation (Σ_{NPQ}) and the sum of fluorescence quenching and constitutive thermal dissipation ($\Sigma_{f,D}$), and the ratio Σ_{PSII}/Σ_A for field grown *Jatropha* leaves (*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 CO₂ 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 $\pm$ SE of 3 values.

Figure 2b. Diurnal time courses of photosynthetic active radiation (PAR), net CO₂ 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 $\pm$ 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 (Φ_{PSII} and F_m/F_v in the dark), quantum yield of basal heat and fluorescence ($\Phi_{f,D}$) and quantum efficiency of thermal dissipation (Φ_{NPQ}) on Feb 19 (pretreatment; left) and Mar 4 (control and water treatment; right). For the different symbols, see Figure 2ab. Values are means $\pm$ SE of 3 values.

Figure 5. Relationship between quantum efficiency of photochemical transport in PSII (Φ_{PSII}) and quantum efficiency of thermal dissipation (Φ_{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

Date	Σ_{PAR} mol m ⁻²	Σ_A mmol m ⁻²	$\Sigma_{f,D}$ mol m ⁻²	Σ_{NPQ} mol m ⁻²	Σ_{PSII} mol m ⁻²	Σ_{PSII}/Σ_A mol mol ⁻¹
Feb 19 (pretreatment)	57.3 ^{a*}	72.4 ^b	4.3 ^a (17.6 ^a)	17.4 ^a (71.7 ^a)	2.6 ^c (10.7 ^c)	37.1 ^a
(control)	55.9 ^a	139.6 ^b	4.4 ^a (17.9 ^a)	16.1 ^{ab} (65.7 ^b)	4.4 ^b (16.3 ^b)	29.1 ^{ab}
Mar 4 (water treatment)	52.5 ^a	330.9 ^a	4.2 ^a (18.2 ^a)	13.1 ^b (56.8 ^c)	5.7 ^a (25.0 ^a)	18.0 ^b

*Tukey-Kramer, $P < 0.05$

Fig 1

Fig 2a

Fig 2b

Fig 3

Fig 4

Fig 5