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# Sago palm (*Metroxylon sagu* Rottb.) response to drought condition in terms of leaf gas exchange and chlorophyll *a* fluorescence

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## ABSTRACT

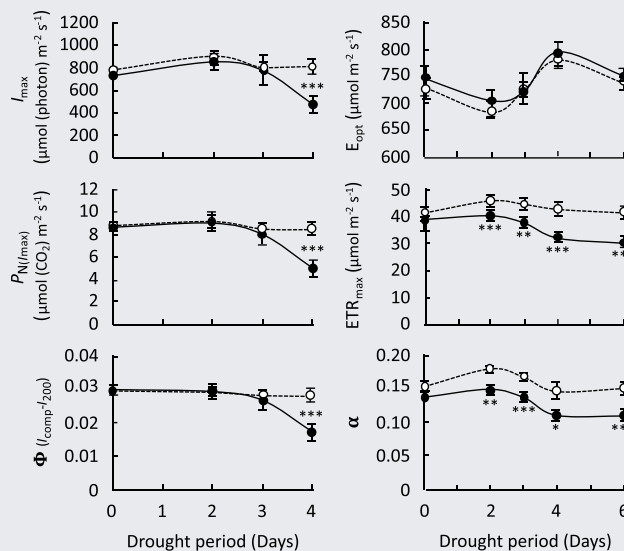
Sago palm (*Metroxylon sagu* Rottb.) photosynthetic performance was evaluated under drought conditions through leaf gas exchange and chlorophyll *a* fluorescence measurement. Drought has an impact on sago palm's leaf gas exchange parameters and its chlorophyll fluorescence of PSII. Stomatal limitations were found to be the main factor limiting photosynthetic activities of sago palm under drought conditions. Photosynthetic rate decreased following the inhibition in the PSII reaction center. Early detection of interference upon the efficiency of the PSII reaction center due to drought stress was recorded by chlorophyll *a* fluorescence measurement.

## ARTICLE HISTORY

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## KEYWORDS

Chlorophyll *a* fluorescence; drought stress; leaf gas exchange; *Metroxylon sagu* Rottb



**Abbreviations:** *CF/I*–light response curve of chlorophyll *a* fluorescence,  $C_i$ –intercellular CO<sub>2</sub> concentration, *E*–transpiration rate,  $E_{opt}$ –optimum irradiance, *ETR*–electron transport rate,  $ETR_{max}$ –maximum electron transport rate,  $g_s$ –stomatal conductance,  $I_{max}$ –maximum irradiance, NPQ–non-photochemical quenching,  $P_N$ –net photosynthetic rate,  $P_{N/I}$ –light response curve of net photosynthetic rate,  $P_{N (I_{max})}$ –maximum value of  $P_N$  obtained at maximum irradiance, WUE–water use efficiency,  $Y(II)$ –effective quantum yield of PSII,  $\alpha$ –asymptotic photosynthetic efficiency,  $\Phi_{(comp-200)}$ –quantum yield at the range between light compensation point and 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  irradiance.

## Introduction

Sago palm occupies 2.2 million hectares in the Malay Archipelago and a part of Melanesia (Flach, 1997). It grows

in swampy, alluvial, and peaty soils where almost no other major crops can grow without drainage or soil improvement (Jong, 1995; Sato et al., 1979). Sago palm is a C3 plant

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in which the initial product of the assimilation of carbon dioxide through photosynthesis is 3-phosphoglycerate, which contains 3 carbon atoms. As with banana and taro, it has long been cultivated as a carbohydrate resource (Takamura, 1990) with potential starch content extractable through pith production estimated at 310 kg per palm (Ehara, 2006). As such sago palm remains a staple food source with potential to produce high yields of starch in problem soils. Consequently, cultivation has expanded from tidal, high soil water levels, wetlands into peat areas that have been artificially drained to regulate the water level for large-scale sago plantations and upland areas where plants depend upon precipitation for water. In certain periods of the year, water availability in these areas may be limited due to low rainfall. A depletion of soil moisture occurs when evapotranspiration is higher than rainfall, increasing tension in the xylem sap trigger stomatal closure and other physiological responses (Wagner et al., 2012).

In general, the physiological activities of plants respond negatively to drought stress, with the degree depending on the duration of the drought and the plant's genetic capacities (Chaves et al., 2002). Plants have been found to respond to drought in several ways: osmotic adjustment, and changes in cell turgidity, stomatal conductance, transpiration, photosynthesis, respiration, antioxidant activity, and light absorption capture (Falqueto et al., 2017; Hsiao et al., 2010; Lawlor & Cornic, 2002; Silva et al., 2013; Velázquez-Márquez et al., 2015).

Among these, photosynthesis is the main process immediately influenced by drought stress (Eberhard et al., 2008). Yet no studies of sago palm photosynthetic performance under water shortage conditions have been reported. As the typical habitat of sago palm is in lowland areas with a high soil water level, this research aimed to use chlorophyll *a* fluorescence to determine the sensitivity of its photosynthetic performance when it

is exposed to the type of water shortage conditions that may be experienced in upland areas.

As a rapid, non-destructive method to monitor plant performance under environmental stress conditions, chlorophyll *a* fluorescence is a useful tool to detect inhibition in photosystem II (PSII) photochemistry due to extended water shortage (Sousa et al., 2014). Chlorophyll *a* fluorescence measurement indicates enhanced protection of PSII and PSI photochemistry under drought stress through adjustment of the energy distribution across the photosystem and by activating alternative electron sinks (Zivack et al., 2013).

In this experiment, we hypothesized that the leaf gas exchange and PSII photochemistry of sago palm would be immediately suppressed under drought stress.

## Materials and methods

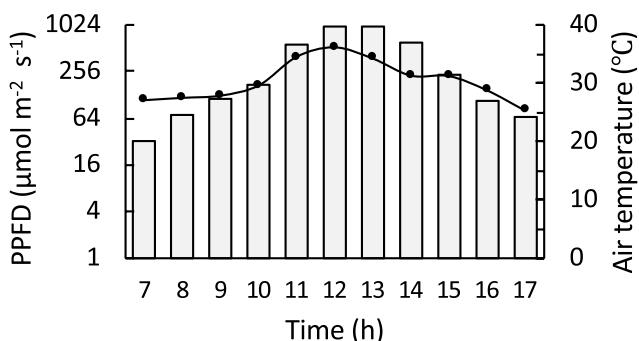
### Experiment location and materials

The experiment was undertaken in an air conditioned glass house (phytotron) at Nagoya University, Japan from April to May, 2018. During the experiment, daytime air temperature ranged from 24 to 36°C, and maximum irradiance flux density inside the phytotron was around 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 1). Average relative humidity was 60% during the day time. The plants were exposed to no artificial light source during the experiment.

Two-year-old sago palm seedlings were grown individually in 5 L pots filled with commercial black soil. Once a week all the plants were watered with 1 liter 50% Kimura B culture solution before drought treatment began. Two different water conditions were applied to the sago seedlings: no water application (drought conditions) and daily water application used as a control. The water application was done every morning until the soil reach a saturated condition. Soil water potential was measured at 10 and 20 cm soil depths from the surface using a tensiometer (Daiki Rika Kogyo Co., Ltd. Saitama, Japan) ( $n = 5$ ). The data was recorded a day before the beginning of drought treatment, on the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> day of drought. All measurements were conducted with the same sago seedlings.

### Leaf gas exchange

Measurements of leaf gas exchange ( $n = 5$ ) such as net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), transpiration rate ( $E$ ) and water use efficiency (WUE) were conducted on the third uppermost leaf position of each plant between 09:00 AM and 01:00 PM using a portable photosynthesis system, Li-6400XT (LiCor Inc., Lincoln, NE, USA) with 6  $\text{cm}^2$  leaf



**Figure 1.** Average diurnal change of photosynthetic photon flux density (PPFD) (bar) and air temperature (line) in phytotron on May 19–22, 2018 in Aichi, Nagoya, Japan.

chamber. WUE was calculated from the ratio of  $P_N$  to  $E$ . The  $\text{CO}_2$  concentration was set at  $400 \mu\text{mol}$  and photosynthetic photon flux density (PPFD) was set at  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The  $\text{CO}_2$  flow rate was adjusted to  $500 \mu\text{mol}$ , relative humidity in the leaf chamber ranged from 50 to 60%, and leaf temperature was set at  $25^\circ\text{C}$ . The measurement was conducted from May 19 to 22 May 2018.

### Light response curve of net photosynthetic rate ( $P_N/I$ )

$P_N/I$  data ( $n = 4$ ) were measured individually on each sago palm leaf sample of all replicates and fitted using a rectangular hyperbola model following the procedure of Lobo et al. (2013). The sequence of light intensity began from 1500, 1250, 1000, 800, 500, 250, 100, 50, 25, and  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The photosynthetic value at each irradiance level was recorded approximately two minutes after the value had stabilized (Azhar et al. 2020). Measurements in several parameters were obtained, including maximum irradiance at the point beyond which no significant change in  $P_N$  occurred ( $I_{\text{max}}$ ), maximum value of  $P_N$  obtained at  $I_{\text{max}}$  ( $P_{N(I_{\text{max}})}$ ), and the linear portion in the curve's slope representing 'the maximum quantum yield' ( $\Phi_{(I_{\text{comp}}-I_{200})}$ ).

### Chlorophyll fluorescence and light response curve of chlorophyll fluorescence (CF/I)

Chlorophyll fluorescence ( $n = 5$ ) was measured using a photosynthesis yield analyzer (MINI-PAM, Heinz Walz, Effeltrich, Germany). The minimum fluorescence in the dark-adapted state ( $F_o$ ) was measured using weak modulated light of  $<0.15 \mu\text{mol m}^{-2} \text{s}^{-1}$  after dark adaptation for 30 minutes. Measuring conditions of chlorophyll fluorescence were set as follows: actinic light:  $55 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; 30 seconds, measuring light:  $0.30 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; 30 micro seconds, and saturation pulse:  $>5500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; 0.8 seconds. Effective quantum yield photosystem II (Y(II)) was calculated as  $Y(II) = (F_m' - F) / F_m'$ . Electron transport rate (ETR) was calculated by  $\text{ETR} = Y(II) \times \text{PAR} \times 0.5 \times \text{ETR-factor}$  (0.84), photochemical quenching (qP) and non-photochemical quenching (NPQ) were defined as  $qP = (F_m' - F) / (F_m' - F_o)$  and  $\text{NPQ} = (F_m - F_m') / F_m'$ . The non-photochemical quenching (NPQ), parameter corresponds to the loss of potential energy through heat dissipation, also referred to as thermodynamic loss.

A rapid light response curve of chlorophyll fluorescence ( $n = 5$ ) was constructed after 30 minutes dark adaptation with nine irradiance levels: 0, 91, 136, 221, 351, 552, 845, 1100 and  $1300 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ , with each irradiance having an interval of 20 seconds. Due to the short time of adaptation between each measurement (20 seconds), it is

possible that photosynthesis of the leaves was not in a steady state at each light intensity. The PAM parameters were calculated with WinControl software (Walz, Effeltrich, Germany) using the standard settings for rapid light curve protocols provided by the Walz soft-ware. The measurement of light-response curve data pairs was conducted and fitted following Ritchie (2008) as described in Azhar et al. (2018). ETR data was fitted using non-linear least squares model calculated as:

$$\text{ETR} = \frac{\text{ETR}_{\text{max}} \times \text{PPFD}}{\text{PPFD}_{\text{opt}}} \times e^{1 - \text{PPFD} / \text{PPFD}_{\text{opt}}}$$

The function in Microsoft Excel for fitting Waiting-in-Line curves was utilized to fit the ETR vs. several levels of irradiance. Several variables derived from the fitted light curve were obtained, including optimum irradiance ( $E_{\text{opt}}$ ) defined as no significant increase in photosynthetic rate with an increase of irradiance, maximum electron transport rate ( $\text{ETR}_{\text{max}}$ ), and asymptotic photosynthetic efficiency ( $\alpha$ ).  $\alpha$  is the initial slope of the curve where at very low light intensities photosynthesis is directly proportional to irradiance.

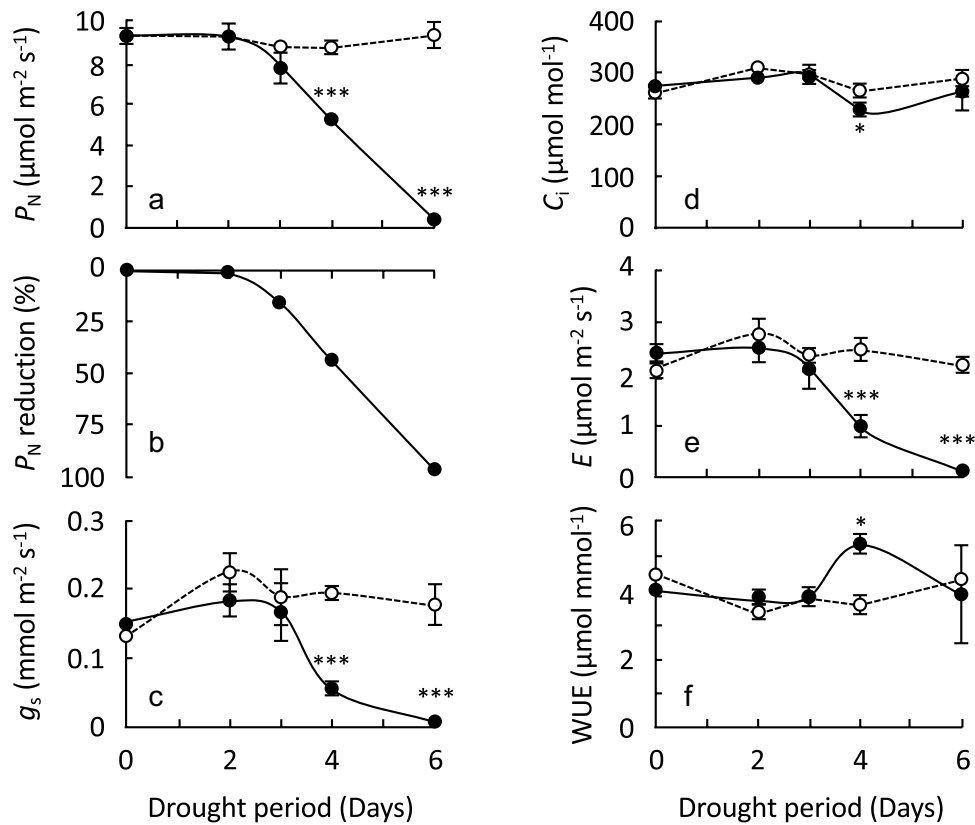
### Statistical analysis

Data from each parameter is presented as mean  $\pm$  SE. To test for differences in all parameters under normal and drought conditions, a Student t-test was performed using MS excel (Microsoft Windows).

## Results

### Leaf gas exchange

The soil water potential value was recorded at  $-5$ ,  $-40$  and  $-70$  kPa on the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> day of drought. Leaf gas exchange values were affected by drought and drought duration. Under drought conditions, reduction of net photosynthetic rate ( $P_N$ ) occurred, accompanied by reduction in stomatal conductance ( $g_s$ ) and leaf transpiration rate ( $E$ ). On the fourth day under drought conditions, the  $C_i$  value was lower than in the control plants. However, by the last day of drought (day 6), the value tended to increase.  $P_N$ ,  $g_s$  and  $E$  were gradually suppressed after three days in the water holding conditions and  $P_N$  sharply decreased by 43.33 and 96.51% between days four and six under drought conditions. The plants tended to maintain water use efficiency (WUE) on the 4<sup>th</sup> day of drought and started a downward trend on the 6<sup>th</sup> day under drought conditions (Figure 2). The results on the light response curve of net assimilation rate ( $P_N/I$ ) revealed that  $I_{\text{max}}$  values trended down after day two of drought conditions and were significantly lower in day four. The reduction trend in  $I_{\text{max}}$  was followed by a



**Figure 2.** Net photosynthetic rate ( $P_N$ ) (a), % reduction in  $P_N$  (b), stomatal conductance ( $g_s$ ) (c), intercellular  $\text{CO}_2$  concentration ( $C_i$ ) (d), leaf transpiration rate ( $E$ ) (e), and water use efficiency (WUE) (f) of sago palm seedlings growing under normal conditions (open circle) and drought conditions (closed circle). \* and \*\*\* indicate significant difference at the 0.05 and 0.001 probability level, respectively according to Student t-test. Data corresponds to the mean value and standard error ( $n = 5$ ).

reduction in  $P_{N(\text{max})}$  and  $\Phi_{(I_{\text{comp}}/I_{200})}$  which gradually decreased with extension of the drought period (Figure 3).

### Chlorophyll fluorescence

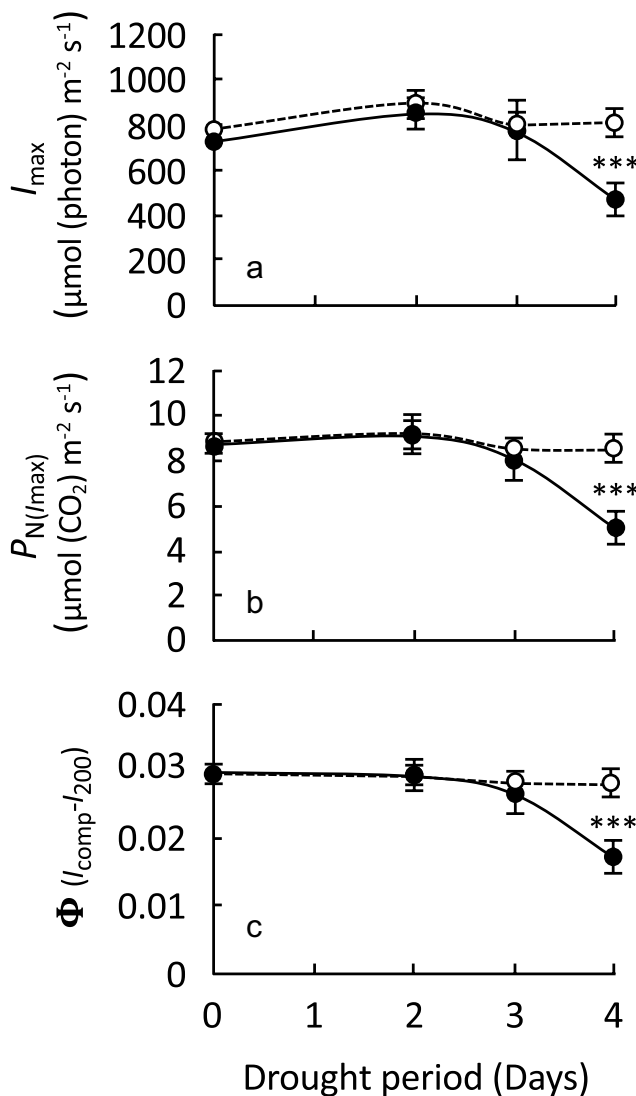
The quantum yield of PSII ( $Y(\text{II})$ ) was not affected by drought conditions. However, drought significantly affected ETR, qP and NPQ values. Drought conditions also caused an increase in  $F_o$  and NPQ values from day 4 to day 6 of the treatment. The  $F_o$  and NPQ increased under drought conditions following a reduction in ETR and qP. The reduction of ETR was detected from the second day under drought conditions, although only slight reduction occurred in the course of the treatment. The qP value followed the same downward trend as ETR. However, significant reduction in the qP value occurred on the last day under drought conditions (Figure 4). No significant effect on optimum irradiance ( $E_{\text{opt}}$ ) for electron transport was found under drought stress. However,  $\text{ETR}_{\text{max}}$  showed a reduction trend when the plants were exposed to drought conditions. The

reduction trend of  $\text{ETR}_{\text{max}}$  was followed by  $\alpha$ . The negative response of  $\alpha$  was detected from day two under drought conditions and constantly decreased over the course of the treatment (Figure 5).

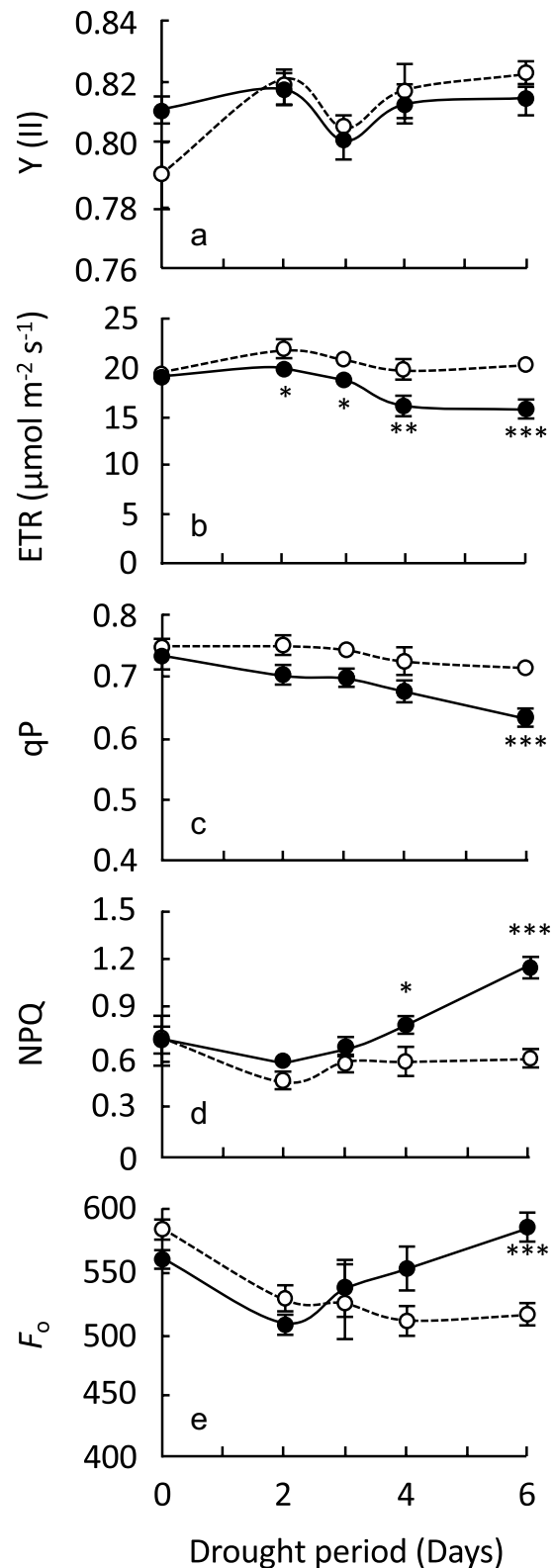
### Discussion

Photosynthesis is affected by stomatal limitations and non-stomatal mechanisms (Damatta et al., 2002). The reduction in stomatal aperture causes an increase in stomatal resistance, which suppresses  $g_s$  and consequently inhibits  $\text{CO}_2$  fixation, contributing to a reduction in net photosynthetic rate (Chaves & Oliveira, 2004). While some studies have assumed that photosynthesis plays a role in  $\text{CO}_2$  regulation of stomatal apertures by lowering the intercellular  $\text{CO}_2$  concentration ( $C_i$ ) in leaves (Roelfsema et al., 2006), other studies indicate that mesophyll cells play a key role by directly sensing  $\text{CO}_2$  (Messinger et al., 2006). In this study, the photosynthetic performance of sago palm under drought stress was mainly affected by stomatal limitations. It showed that under drought conditions, reduction of leaf gas

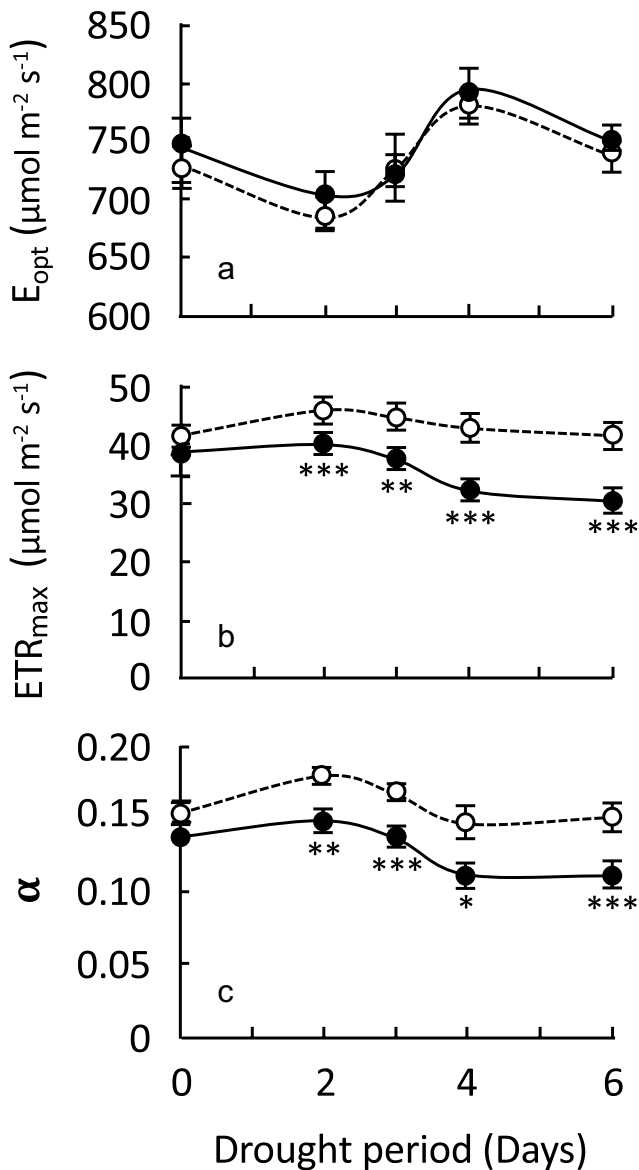
exchange parameters and  $P_N/I$  began after 3 days, declining to zero by day 6 of water deficit. However, intercellular  $\text{CO}_2$  concentration ( $C_i$ ) did not show a direct response to net photosynthetic rate ( $P_N$ ) and stomatal conductance ( $g_s$ ). Stomatal conductance does not always respond to intercellular  $\text{CO}_2$  as mesophyll tissue in leaves plays an important role in  $\text{CO}_2$  sensing (Mott, 2009). According to Flexas et al. (2008) and Terashima et al. (2011), the level of  $\text{CO}_2$  diffusion resistance in the mesophyll ( $r_m$ ) will correspond to that of  $\text{CO}_2$  diffusion resistance through the stomata ( $r_a+r_s$ ). In this experiment,  $g_s$  decreased in the treated plants, however, the extent of  $C_i$  decrease in the treated plants was comparatively small compare to  $g_s$ . Miyazaki and The Society of Sago Studies (2015) supposes that photosynthesis may be mainly controlled by the stomatal factor based on the fact that the extent of rate-limiting by the stomata was



**Figure 3.** Changes in  $I_{\max}$  (a),  $P_{N(I_{\max})}$  (b),  $\Phi(I_{\text{comp}}-I_{200})$  (c) of sago palm seedlings in normal conditions (open circle) and under drought conditions (closed circle). \*\*\* indicates significant difference at the 0.001 probability level according to Student t-test. Data corresponds to the mean value and standard error ( $n = 4$ ). Data of Figure a, b, and c are variables obtained from  $P_N/I$ .



**Figure 4.** Effectively quantum yield photosystem II (Y(II)) (a), electron transport rate (ETR) (b), photochemical quenching coefficient (qP) (c), non-photochemical quenching (NPQ) (d), minimum chlorophyll fluorescence yield in the dark-adapted state ( $F_0$ ) (e) of sago palm seedlings growing under normal conditions (open circle) and drought conditions (closed circle). \*, \*\* and \*\*\* indicate significant difference at the 0.05, 0.01 and 0.001 probability level, respectively according to Student t-test. Data corresponds to the mean value and standard error ( $n = 5$ ). Irradiance flux density during measurement range from 600 to 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .



**Figure 5.** Changes in  $E_{opt}$  (a),  $ETR_{max}$  (b),  $\alpha$  (c) of sago palm seedlings in normal conditions (open circle) and under drought conditions (closed circle). \*, \*\* and \*\*\* indicate significant levels at the 0.05, 0.01 and 0.001 probability level, respectively according to Student t-test. Data corresponds to the mean value and standard error ( $n = 5$ ). Data of Figure a, b, and c are variables obtained from  $CF/I$ .

substantial (30% or more) because the transpiration rate and the stomatal density were low at the seedling stage (Uchida et al., 1990) and the photosynthesis rate after trunk formation showed a strong correlation with mesophyll conductance rather than stomatal conductance (Miyazaki et al., 2007). Although we did not measure  $r_a + r_s$  and  $r_m$  directly, considering these results,  $r_m$  might decrease as per the decrease in  $r_a + r_s$ .

The reduction in the non-stomatal limitation mechanisms of the light harvesting system center was not as severe as the reduction in the stomatal limitation. This

suggests photosystem II is more resistant to water deficit. However, negative impact on PSII has previously been found to occur under prolonged water shortage conditions leading to extreme drought (Lauriano et al., 2006).

The results for  $P_N/I$  and  $CF/I$  indicated that drought negatively affected sago palm's leaf gas exchange parameters and chlorophyll *a* fluorescence PSII photochemistry. Nevertheless, the immediate response of plants to drought stress was detected by chlorophyll fluorescence measurement. In the early stage of drought, the reduction of  $CO_2$  uptake due to stomatal closure might be partially responsible for a reduction in leaf photosynthesis. Concerning the physiological response of sago palm under salt stress, Ehara et al. (2008) reported that the stomatal aperture might be small and lead to a decrease in the photosynthetic rate; however, the remarkable decrease in the transpiration rate with NaCl treatment could be interpreted as one of the mechanisms maintaining the water status in the plant body by restricting the water loss in sago palm. As Pirasteh-Anosheh et al. (2016) explained, although  $CO_2$  assimilation rate and net photosynthesis are reduced due to stomatal closure, attainment of a low transpiration rate and inhibition of water loss from leaves can be a useful trade-off, in exchange for growth, serving survival. Since stomatal closure has negative effects on  $CO_2$  uptake, photosynthesis, transpirational cooling, as well as water and nutrient uptake, it is important to close the stomata only when the benefit of water retention outweighs the negative effects (Pirasteh-Anosheh et al., 2016). Under water shortage conditions the reduction of the stomatal aperture within a certain range could also be recognized as the result of a trade-off for water retention for a while after the sago palm has faced drought conditions. Further investigation of the relationship between stomatal closure and water status in the plant body of the sago palm may provide better understanding of the role of stomata in growth performance under conditions of comparatively low water supply.

Non-invasive indicators provided by chlorophyll fluorescence can tell us the status of the photosynthetic reaction center in harvesting light energy (Calatayud et al., 2006). Under drought conditions, disturbance in ETR was detected from the 2<sup>nd</sup> day of drought treatment (Figure 4b). The allocation of light energy for photochemical quenching (qP) was also suppressed under drought conditions as more irradiance was dissipated as NPQ. The reductions in ETR and qP values were associated with an increase in  $F_o$  which began on the 3<sup>rd</sup> day under drought conditions and had significantly increased by day 6 (Figure 4e). The increase of  $F_o$  is

related to reduction in the efficiency of energy trapping in PSII which consequently inhibits the electron flow from  $Q_A$  to  $Q_B$  in PSII (Falqueto et al., 2017). The increase in  $F_o$  is also associated with rapidly reversible damage to the D1 protein linked to PSII (Strasser & Strasser, 1995).

The above results for  $P_N/I$  and  $CF/I$  clearly indicated that other photosynthetic processes likely work in conjunction with the co-limit  $P_N$  in drought-stressed sago palm. Further understanding of sago palm photosynthetic performance was gleaned by analyzing  $P_N/I$  and  $CF/I$  (Figs. 3 & 5). Photosynthetic performance was monitored from before the introduction of drought conditions until the 4<sup>th</sup> day.  $P_N$  had decreased by 96.51% by day 6. The negative effect of drought on photosynthetic ability was reflected in the reduction of light saturation point ( $I_{max}$ ).  $I_{max}$  is the point above which an increase in photon flux produces no further increase in photosynthesis. After the saturation point, photosynthesis is referred to as  $CO_2$  limited, due to the inability of the Calvin cycle enzymes to keep pace with the absorbed light energy. A reduction of  $I_{max}$  leads to reduction in net photosynthetic rate at maximum absorbed irradiance ( $P_{N(I_{max})}$ ). It indicates an inhibition of maximum light energy utilization for  $CO_2$  fixation. Through  $CF/I$  measurement, we found that the light saturation point for electron transport ( $E_{opt}$ ) was not influenced by drought with in the time-frame of this experiment. However, the electron transport rate at maximum irradiance ( $ETR_{max}$ ) was significantly lower under drought conditions, although the reduction was not as low as  $P_{N(I_{max})}$ .

Drought stress also caused interference to the efficiency of light energy utilization for  $CO_2$  fixation and electron transport of sago palm.  $\Phi_{(I_{comp}-I_{200})}$  is the linear portion of the  $P_N/I$  curve in the range of approximate light compensation point to 200  $\mu\text{mol}$  (photon)  $\text{m}^{-2} \text{s}^{-1}$ , representing the 'maximum quantum yield'. The light compensation point is the value of irradiance where  $CO_2$  fixed by photosynthesis is equal with the  $CO_2$  released through light respiration and photorespiration, resulting in  $P_N$  equaling zero. A supposed linear response of  $P_N$  to irradiance is obtained when irradiance reaches approximately 200  $\mu\text{mol}$  (photon)  $\text{m}^{-2} \text{s}^{-1}$  (Lobo et al., 2013).  $\alpha$  is the condition where photosynthesis is directly proportional to irradiance. It also explains the maximum photosynthetic efficiency at the initial slope of the curve at very low irradiance intensities (Ritchie, 2008).

In conclusion, stomatal limitations were the main factor limiting photosynthetic activities of sago palm under drought conditions. However, early interference upon the efficiency of the PSII reaction center due to drought stress was recorded by chlorophyll fluorescence measurement. In addition, the light response curve of chlorophyll *a* fluorescence ( $CF/I$ ) provided more information on the optimal

and efficient light energy utilization for photosynthesis. Photosynthetic inhibition may cause a reduction in starch accumulation in the trunk of sago palm. However, further experimentation using sago palm growing in the field is needed, as photosynthesis may show a different response to that in the pot experiment due to the roots being better able to access water in a deeper soil layer.

## Disclosure statement

The authors declare no conflict of interest.

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