

Article

# Photosynthesis of Sago Palm (*Metroxylon sagu* Rottb.) Seedling at Different Air Temperatures

Aidil Azhar <sup>1,2,\*</sup>, Daigo Makihara <sup>3</sup>, Hitoshi Naito <sup>4</sup> and Hiroshi Ehara <sup>3,5,\*</sup> 

<sup>1</sup> Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-0814, Japan

<sup>2</sup> Vocational School, Bogor Agricultural University, Bogor 16680, Indonesia

<sup>3</sup> International Cooperation Center for Agricultural Education, Nagoya University, Nagoya 464-0814, Japan; makihara@agr.nagoya-u.ac.jp

<sup>4</sup> College of Life Science, Kurashiki University of Science and The Arts, Kurashiki 712-8505, Japan; naito@sci.kusa.ac.jp

<sup>5</sup> Applied Social System Institute of Asia, Nagoya University, Nagoya 464-0814, Japan

\* Correspondence: aidilazhars@gmail.com (A.A.); ehara@agr.nagoya-u.ac.jp (H.E.);

Tel.: +81-52-789-4232 (H.E.)

Received: 27 October 2017; Accepted: 20 December 2017; Published: 1 January 2018

**Abstract:** Photosynthetic activities of the sago palm (*Metroxylon sagu* Rottb.) were studied to find out its sensitivity to changes in ambient air temperature. The minimum ambient air temperature designed for the experiment was 25–29 °C, while the higher end was 29–33 °C. Several photosynthetic parameters were studied to support our analysis in sago photosynthetic activity, including diurnal leaf gas exchange, assimilation rate vs. CO<sub>2</sub> concentration, leaf greenness, leaf chlorophyll content, and photosynthetic rate vs. irradiance. We found that sago palm photosynthetic activity tends to be more sensitive to minimum than to maximum ambient air temperature. The plants exposed to higher air temperatures had dark green leaf color associated with higher rates of diurnal photosynthesis, chlorophyll content, and rubisco limited photosynthetic activity. They also exhibited higher trend in optimum irradiance absorption level. Consequently, maximum light energy dissipation occurred at higher temperatures.

**Keywords:** carbon response curve; light response curve; photosynthesis; pigment determination; sago palm

## 1. Introduction

Ambient air temperature generally has a significant impact the physiological performance of plants. Many studies reveal that inhibition of photosynthetic performance occurs at severely high (>35 °C) and low temperatures (<20 °C) [1]. However, in the tropical zone in which the sago palm typically grows, variation in air temperature within a year is less than in temperate zones, so this study focused on a moderate air temperature range from 25–33 °C to ascertain the sago palm's photosynthetic performance in the ambient air environment of its typical habitat.

The sago palm is a perennial monocot crop well known for its potential to accumulate high amounts of starch in its trunk. It can store approximately 300 kg (dry weight) of starch per tree [2]. The importance of sago palm as a staple food is well recognized in some areas of Southeast Asia and the South Pacific. The carbohydrate contained in the trunk can be further processed into various basic raw materials for food, animal feed, and industrial uses [3]. Coming from the Arecaceae family, sago is able to grow in marginal terrain such as submerged and tidal areas where most agronomy crops cannot survive without drainage or soil improvement. As one of the most important crops for sustainable agriculture and for rural development in swampy areas of Indonesia, the sago palm has become an important part of peatland restoration projects.

The optimum air temperature range for the sago palm is very narrow, reported as from 25 °C to 29 °C [4]. Elsewhere, it has been reported that the best growing conditions required a minimum of at least 26 °C [5]. The minimum temperature has been cited as an important factor limiting sago palm performance [6], which is likely due to the tendency of the photosynthesis enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) to be very sensitive to air temperature. Minimum temperature reduces rubisco activity, which consequently reduces the utilization of RuBP by rubisco. Rubisco activase content is suppressed by up to 80% at 25 °C and below [7]. A study on hibiscus plants reported that the effective quantum yield of PSII ( $\Phi_{\text{PSII}}$ ) is suppressed below 10 °C [8].

Regarding maximum temperature, morphological observation of sago palm seedlings reported that at 35 °C leaves expanded but become less green [9]. Higher air temperature also plays an important role in sago seed germination. At 30 °C, seed germination was about 20% higher than at 25 °C [10]. In physiological studies, moderate heat stress affects light energy harvesting as more light is dissipated for non-photochemical quenching rather than photochemical quenching. Therefore, the CO<sub>2</sub> fixation process is depressed [11]. Another study confirmed that carboxylation efficiency decreased at 39 °C followed by a reduction in photosynthetic efficiency [12].

While it has been considered that variations of ambient air temperature in the sago palm habitat might affect its physiological performance, especially its photosynthetic activity, this has not been definitively proven. This study aimed to provide useful data on sago palm physiology at different air temperatures beyond the little that has so far been published. Understanding the regulation of photosynthesis and chlorophyll fluorescence is very important as a tool in characterizing plant reactions under abiotic stress, such as high and low temperature stress [13]. Thereby, the area for sago palm cultivation can be effectively selected to meet the need for appropriate air temperature, allowing optimum plant growth to be achieved and producing optimal yields.

Due to the abovementioned high degree of photosynthetic sensitivity of the plant, especially at lower temperatures, it was hypothesized that even moderate changes in air temperature will inhibit the photosynthetic performance of sago palm.

## 2. Materials and Methods

### 2.1. Plant Material and Culture Conditions

The experiment was conducted in two phytotrons (air conditioned glass house) with air temperatures ranging from 25–29 °C and 29–33 °C respectively at Nagoya University, Japan, from January to March 2017. These air temperatures were considered as the range of ambient temperature in sago palm habitats associated with tropical rainforest climate. Air relative humidity ranged from 30–50% and irradiance flux density from 600–800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Six one-year-old sago palm seedlings with six fully-developed leaves, grown individually in 1/10000a Wagner pots (diameter 115 mm and height 184 mm), were tested. The plant materials were obtained in seed form from Sentani District, Jayapura, Indonesia. Vermiculite was applied as the growing media for each plant. The nutrients were supplied through the application of Kimura B culture solution. The second youngest leaves were selected for all measurements. At the beginning, all plants were placed in the same phytotron with air temperature set at 25–29 °C. After that, six plants were moved to a phytotron with air temperature at 29–33 °C. After one month of acclimation, measurement was conducted.

### 2.2. Diurnal Leaf Gas Exchange

Diurnal leaf gas exchange was measured hourly from 7:00 AM to 5:00 PM. The diurnal leaf gas exchange measurement was ended at 3:00 PM to the plants grown at 25–29 °C as the photosynthetic value was zero after 3:00 PM. The portable photosynthesis system, Li-6400XT (LiCor Inc., Lincoln, NE, USA) with 6 cm<sup>2</sup> leaf chamber was utilized during the measurement. The CO<sub>2</sub> concentration was controlled at 400  $\mu\text{mol}$  and photosynthetic photon flux density (PPFD) was set at 750  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The CO<sub>2</sub> mixer was adjusted at 500  $\mu\text{mol}$ , and relative humidity in the leaf chamber was controlled at 40%.

During measurement, leaf temperature was set at 25 °C. Net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $T_r$ ), and intercellular  $CO_2$  concentration ( $C_i$ ) parameters were obtained from this measurement.

### 2.3. Assimilation Rate vs. $CO_2$ Concentration ( $A/C_i$ Curve)

A carbon response curve was constructed following the Farquhar photosynthesis model [14,15] to conceive the photosynthesis interference at different air temperatures. Changes in rates of assimilation in response to carbon dioxide variation was studied by setting several levels of  $CO_2$  concentration with constant PPFD intensity set at 750  $\mu\text{mol}$ . At the beginning,  $CO_2$  concentration was set at 400  $\mu\text{mol}$  and gradually reduced to the lowest concentration at 50  $\mu\text{mol}$ . After reaching the lowest level,  $CO_2$  concentration was gradually increased to a maximum level of 2000  $\mu\text{mol}$ . Finally,  $CO_2$  concentration was returned to 400  $\mu\text{mol}$  with irradiance in the “off” mode. There were three replicates for each treatment.

The  $A/C_i$  curves data were obtained by  $A/C_c$  curve fitting utility version 1.1 developed by Sharkey [15]. The derived variables obtained from the fitting curve are maximum carboxylation capacity ( $V_{cmax}$ ), and electron transport rate ( $J$ ) [16]. The Rubisco limited photosynthesis ( $V_{cmax}$ ) was calculated using the following equation:

$$A = V_{cmax} \left[ \frac{C_c - \Gamma^*}{C_c + K_c (1 + O/K_o)} \right] - R_D \quad (1)$$

$V_{cmax}$  represents maximum Rubisco rate in  $CO_2$  reduction,  $C_c$  is partial  $CO_2$  pressure at rubisco,  $K_c$  is the Michaelis constant of Rubisco for  $CO_2$ ,  $O$  is the partial pressure of  $O_2$  at rubisco,  $K_o$  is the inhibition constant of Rubisco for  $O_2$ ,  $\Gamma^*$  is the compensation point of photorespiration, and  $R_D$  is dark respiration in which  $CO_2$  is released by the non-photorespiration process.

The following equation was used to calculate the RuBP limited photosynthesis:

$$A = J \frac{C_c - \Gamma^*}{4 C_c + 8 \Gamma^*} - R_D \quad (2)$$

$J$  represents the RuBP limited photosynthesis for NADPH formation, which is utilized in RuBP regeneration, which takes four electrons per carboxylation and oxygenation [15].

### 2.4. Photosynthetic Rate vs. Irradiance

A light response curve was constructed using a photosynthesis yield analyzer (MINI-PAM, Walz-Effeltrich, Germany) after dark adaptation for 20 min. Nine irradiance levels were given from zero to 1300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and each irradiance had an interval of 10 s to reach to steady state level. Three sago seedlings were chosen as replicates. Three leaflets from the second younger leaf of each plant were measured to obtained the mean value of each replicate.

The fluorescence data including quantum yield photosystem II ( $\Phi_{PSII}$ ), electron transport rate (ETR), non-photochemical quenching (NPQ), and coefficient of non-photochemical quenching (qN), were computed with WinControl software (Walz-Effeltrich, Germany). The fraction of energy photo-chemically converted in photosystem II is represented by  $\Phi_{PSII}$ , which is calculated as

$$\Phi_{PSII} = \frac{F_m' - F}{F_m'} = \frac{\Delta F}{F'} \quad (3)$$

$F_m'$  is the maximum fluorescence yield in light adapted sample where all PSII is the open stage,  $F$  is yield fluorescence measured briefly before saturation pulse application, and  $\Delta F$  is the increase of fluorescence induced by a saturation pulse [17]. The equation used to fit  $\Phi_{PSII}$  is simple exponential decay function of the form  $\Phi_{PSII} = e^{-x}$  after appropriate scaling,

$$\Phi_{PSII} = \Phi_{PSII \max} \times e^{-k_y \text{ PPFD}} \quad (4)$$

$\Phi_{\text{PSII}}$  is quantum yield,  $\Phi_{\text{PSIImax}}$  is maximum quantum yield at theoretical zero irradiance,  $k_y$  is a scaling constant, and PPFD is photon flux density ( $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ).

Electron transport rate (ETR) is calculated by estimating gross photosynthesis using the following equation:

$$\text{ETR} = \Phi_{\text{PSII}} \times \text{PPFD} \times 0.5 \times 0.84 \quad (5)$$

$\Phi_{\text{PSII}}$  is the effective quantum yield, PPFD is the irradiance, allocation factor (0.5) is the partitioning energy between PS II and PS I, and 0.84 is the leaf absorbance factor ( $\alpha_{\text{leaf}}$ ) [18]. Following Ritchie and Bunthawin [19], ETR data was fit using non-linear least squares methods calculated as

$$\text{ETR} = \frac{\text{ETR}_{\text{max}} \times \text{PPFD}}{\text{PPFD}_{\text{opt}}} \times e^{1-\text{PPFD}/\text{PPFD}_{\text{opt}}} \quad (6)$$

The excel routine for fitting Waiting-in-Line curves was utilized to fit the ETR vs. several levels of irradiance [20]. The excel routine was obtained personally from Ritchie. The non-photochemical quenching (NPQ) parameter corresponds to the loss of potential energy which is dissipated as heat also referred to as thermodynamic loss. NPQ is calculated as

$$\text{NPQ} = \frac{Y(\text{NPQ})}{Y(\text{NO})} = \frac{F_m - F_m'}{F'} \quad (7)$$

qN is calculated as

$$qN = \frac{F_m - F_m'}{F_m - F_0} \quad (8)$$

$F$  is yield fluorescence measured briefly before saturation pulse application,  $F_m$  is the maximum fluorescence of dark adapted leaf,  $F_0$  is the minimum fluorescence, and  $F_m'$  is the maximum fluorescence measured at saturation pulse. The equation used to fit qN and NPQ vs. irradiance curves is simple exponential saturation functions.

$$qN = qN_{\text{max}} [1 - \exp(-K_{qN} \times \text{PPFD})] \quad (9)$$

While NPQ was calculated as

$$\text{NPQ} = \text{NPQ}_{\text{max}} [1 - \exp(-K_{\text{NPQ}} \times \text{PPFD})] \quad (10)$$

PPFD is photon flux density,  $K_{qN}$  and  $K_{\text{NPQ}}$  are exponential constants,  $qN_{\text{max}}$  is the asymptotic maxima for qN, and  $\text{NPQ}_{\text{max}}$  is the asymptotic maxima for NPQ.

## 2.5. Chlorophyll Content

A portable chlorophyll meter, SPAD-502Plus (Konica Minolta-Kyoto, Japan), was utilized to measure leaf greenness of the same leaves chosen for photosynthesis measurement. The same leaves were harvested for chlorophyll content analysis.

Chlorophyll determination was conducted following Arnon [21] and Lichtenthaler [22] after acetone 80% extraction using a spectrophotometry (UV-1800 Shimadzu-Kyoto, Japan). Chlorophyll and carotenoids concentrations were calculated in  $\mu\text{g g}^{-1}$  ground sample.

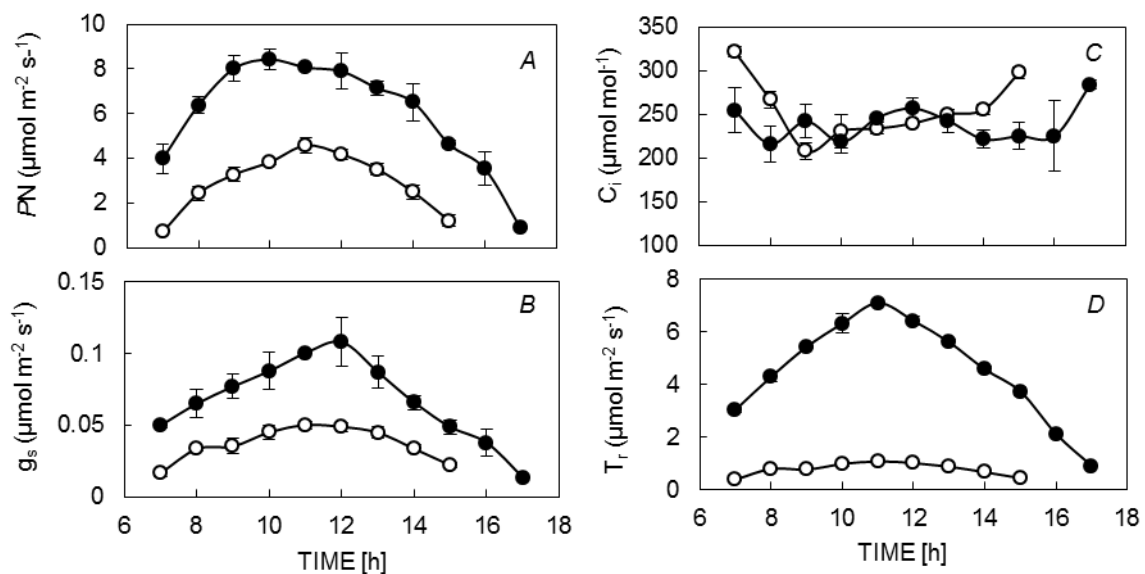
## 2.6. Statistical Analysis

This experiment employed completely randomized design with two ranges of air temperature, 25–29 °C and 29–33 °C, with three replicates. The second upper-most leaf from each replicate was used for measurement. Each datum is presented as mean  $\pm$  SE. To test for differences of photosynthetic rate and other supporting parameters at two air temperature ranges, Student's *t*-test were performed using MS excel.

### 3. Results

#### 3.1. Diurnal Leaf Gas Exchange

Data for the sago palm show considerable differences in net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), and leaf transpiration rate ( $T_r$ ) values between the two air temperature ranges (Figure 1). The open circles represent the  $P_N$  of sago palm seedlings growing at 25–29 °C room temperature, while the closed circles represent the  $P_N$  of seedlings grown at 29–33 °C room temperature. In the first daily measurement at 7:00 h, sago seedlings growing at the higher room temperature started with a higher  $P_N$  than the seedlings growing at lower temperature. The plants grown at 25–29 °C maintained the optimum  $P_N$  for a short period as the down-ward trend began around 12:00 h.



**Figure 1.** Diurnal change in (A) net photosynthetic rate ( $P_N$ ); (B) stomatal conductance ( $g_s$ ); (C) intercellular  $CO_2$  concentration; (D) transpiration rate ( $T_r$ ) of sago palm seedlings growing at different room temperatures. ○: 25–29 °C, ●: 29–33 °C. (Mean  $\pm$  SE,  $n = 3$ ).

Stomatal conductance ( $g_s$ ) and leaf transpiration rate ( $T_r$ ) showed the same trend with  $P_N$ . At 25–29 °C,  $g_s$  showed a slight upward trend during the observation. Low stomatal aperture at 25–29 °C caused a reduction in  $T_r$  (Figure 1B,D). At 29–33 °C, the higher  $P_N$  trend was followed by higher  $g_s$  and  $T_r$ . However, intercellular  $CO_2$  concentration ( $C_i$ ) at higher temperature showed a lower rate only in the first two hours of measurement (7:00 to 8:00 h) and the last two hours of measurement. They exhibited almost the same trend in  $C_i$  rate between 9:00 and 13:00 h (Figure 1C).

#### 3.2. Photosynthetic Activity and Chlorophyll Content

Photosynthetic activity and chlorophyll content of sago palm seedlings showed lower rates and concentrations respectively at 25–29 °C air temperature. The low  $P_N$  value was followed by lower values in biochemical limiting photosynthetic activities such as electron transport rate ( $J$ ) and maximum carboxylation capacity ( $V_{cmax}$ ). The other parameters to explained the low  $P_N$  at minimum air temperature was pigmentation. Chlorophyll content seems to be lower at minimum than at higher air temperatures tested. Although only Chl *b* showed significant difference in pigment content, all parameters were considerably higher in sago seedlings grown at 29–33 °C air temperature (Table 1).

**Table 1.** Photosynthetic parameters and chlorophyll content of sago palm seedlings at different air temperatures.

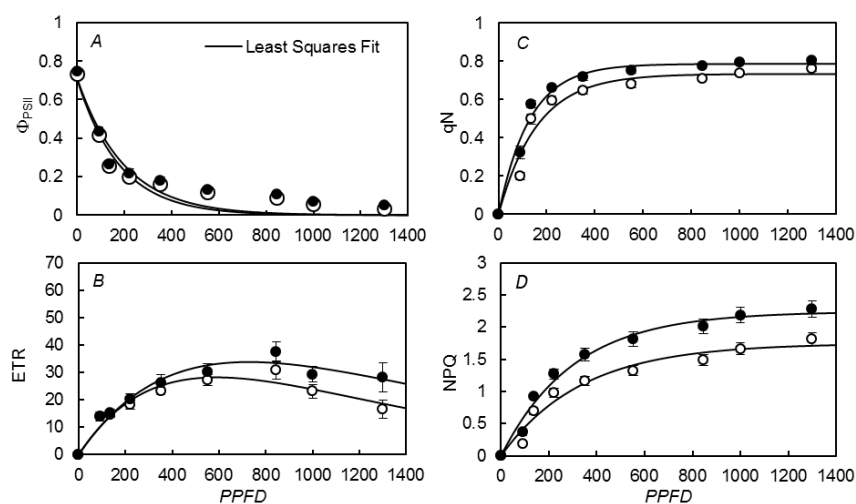
Parameters	25–29 °C	29–33 °C
$P_N$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	$5.66 \pm 0.91$	$8.62 \pm 0.24^*$
$J$ [ $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ ]	$88.2 \pm 10.98$	$114.1 \pm 5.59^{\text{ns}}$
$V_{\text{cmax}}$ [ $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ ]	$40.1 \pm 10.23$	$99.3 \pm 10.86^*$
SPAD	$46.2 \pm 1.56$	$62.3 \pm 1.91^{**}$
Chl $a$ [ $\mu\text{g} \text{g}^{-1}$ ]	$838.1 \pm 34.39$	$987.3 \pm 99.04^{\text{ns}}$
Chl $b$ [ $\mu\text{g} \text{g}^{-1}$ ]	$258.5 \pm 10.56$	$319.2 \pm 11.45^*$
Chl $a + b$ [ $\mu\text{g} \text{g}^{-1}$ ]	$1096.6 \pm 35.9$	$1306.5 \pm 102.8^{\text{ns}}$
Carotenoid [ $\mu\text{g} \text{g}^{-1}$ ]	$223.7 \pm 2.91$	$255.0 \pm 2.91^{\text{ns}}$

\*: significant ( $p \leq 0.05$ ), \*\*: significant ( $p \leq 0.01$ ), <sup>ns</sup>: not significant ( $p > 0.05$ ), respectively using Student  $t$ -test. Means  $\pm$  SE,  $n = 3$ .

The leave color of sago palm grown at 29–33 °C was obviously darker than at the lower air temperature tested and was confirmed by significantly higher SPAD values. In addition, we noticed that most plants showed leaf emergence at the higher air temperature, although no measurement in leaf emergence rate was undertaken.

### 3.3. Photosynthetic Rate vs. Irradiances

Photosynthetic rate vs. irradiances was measured and fit using non-linear least square fit. The graphics of quantum yield of PSII at both air temperatures were obtained (Figure 2A). The curve of quantum yield of PSII ( $\Phi_{\text{PSII}}$ ) vs. irradiance at both air temperatures provided a maximum effective quantum yield ( $\Phi_{\text{PSIImax}}$ ) value at 25–29 °C and 29–33 °C. The Waiting-in-Line equation was used to fit ETR values (Figure 2B) for both air temperature ranges tested. The variables obtained PPFD<sub>opt</sub>, ETR<sub>max</sub> and  $\alpha_0$  are shown in Table 2. The fitted qN and NPQ data (Figure 2C,D) provide information about qN<sub>max</sub> and NPQ<sub>max</sub>. Most of variables in Table 2 are dominated by the sago seedlings growing at 29–33 °C air temperature. However, according to statistical analysis, there are no significant differences in maximum efficiency quantum yield ( $\Phi_{\text{PSIImax}}$ ), maximum electron transport rate (ETR<sub>max</sub>), asymptotic photosynthetic efficiency ( $\alpha_0$ ), and maximum coefficient of non-photochemical quenching (qN<sub>max</sub>) between the two air temperature ranges.



**Figure 2.** (A) Photosynthetic yield ( $\Phi_{\text{PSII}}$ ); (B) electron transport rate (ETR); (C) coefficient non-photochemical quenching (qN); and (D) non-photochemical quenching (NPQ) vs. irradiance of sago palm seedlings (estimated via non-linear least square fitting) at 25–29 °C (○) and 29–33 °C (●). Mean  $\pm$  SE,  $n = 3$ , with 9 irradiance levels.



**Table 2.** Photosynthetic parameters (fitted by Waitting-in-Line equation) of sago palm seedling at 25–29 °C and 29–33 °C air temperatures.

Parameters	25–29 °C	29–33 °C
Maximum yield ( $\Phi_{PSII_{max}}$ )	0.70 ± 0.013	0.70 ± 0.025 <sup>ns</sup>
Optimum PPFD ( $PPFD_{opt}$ )	587.00 ± 29.81	725.59 ± 33.32 *
Maximum electron transport rate ( $ETR_{max}$ )	28.28 ± 2.53	33.91 ± 3.76 <sup>ns</sup>
Asymptotic Photosynthetic efficiency ( $\alpha_o$ )	0.13 ± 0.006	0.13 ± 0.009 <sup>ns</sup>
$qN_{max}$	0.73 ± 0.015	0.79 ± 0.014 <sup>ns</sup>
$NPQ_{max}$	1.75 ± 0.08	2.25 ± 0.13 *

\*: significant ( $p \leq 0.05$ ), <sup>ns</sup>: not significance ( $p > 0.05$ ) as the results of Student *t*-test. Means ± SE,  $n = 3$  plants, 27 data points.

#### 4. Discussion

At the beginning of measurement (7:00 h) sago palm seedlings revealed low photosynthetic rates in both treatments. This might be due to the light not reaching sufficient levels for optimum stomatal aperture as blue light induces the aperture of stomata [23]. Moreover, air temperature at that time has not reached the optimum level for higher rubisco activity. Consequently, with an increase in light intensity and air temperature from 8:00–11:00 h,  $P_N$ ,  $g_s$ , and  $T_r$  trended upward in both treatments. However, the plants showed midday depression as photosynthetic rate reduced from 12:00 h. This might be caused by stomatal and other non-stomatal limitations such as photoinhibition, photorespiration, and reduction of rubisco activity under high temperature [24]. This is consistent with most field work cases, where it is difficult to obtain optimum  $P_N$  rate in sago palm when the measurement is conducted after 12:00 h. This information was confirmed by our findings from diurnal leaf gas exchange data. In addition, our data suggests that lower air temperature inhibited the seedlings' capacity to maintain a longer  $P_N$  rate. Higher temperatures (29–33 °C) appear to induce higher rubisco activity in sago palm seedlings than that achieved at lower temperatures (25–29 °C) (Table 1). Although producing the same diurnal leaf gas exchange trend across both air temperature ranges, the sago seedlings growing at 29–33 °C room temperature showed higher photosynthetic rate.

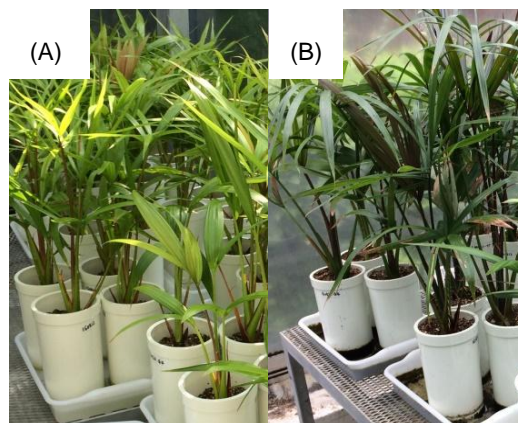
According to the data from Figure 1A, a lower net photosynthetic rate at 25–29 °C was followed by lower stomatal conductance results in lower leaf transpiration rate. Low transpiration rate is considered to be one of the factors causing low  $P_N$  rate as  $CO_2$  can only enter leaves through gas diffusion [23]. However, the intercellular  $CO_2$  concentration ( $C_i$ ) did not show a higher rate at 25–29 °C.  $C_i$  revealed almost the same trend in both air temperature ranges, except in the first two hours of measurement and the last two hours of measurement (Figure 1C). When plants performed higher assimilation rates, intercellular  $CO_2$  value should show a lower rate as the  $CO_2$  is utilized during photosynthesis activity. Therefore, we assume that at 25–29 °C, the photosynthetic activity was not only limited by those components but also the other components such as rubisco activity, leaf chlorophyll content and the light harvesting system.

In the lower air temperature range (25–29 °C), the performances of rubisco activity ( $V_{cmax}$ ) tend to be low. Low rubisco activity at 25 °C might be due to the reduction in RuBP regeneration [7,25]. RuBP regeneration might be affected by the lower RuBP consumption by rubisco. This could suggest that the activation state of rubisco could be different in plants grown at different air temperatures.

The higher performance in net photosynthetic rate of sago palm seedlings at higher temperatures tested also could not be separated from the support of higher photosynthetic apparatus formation, such as leaf chlorophyll. The higher temperatures induced higher formation of leaf pigments such as Chl *a*, Chl *b*, and carotenoid. The sago seedlings grown at higher air temperatures produced leaves with a dark green color, while sago seedlings grown at lower air temperature produced light green leaves (Figure 3). An appropriate air temperature increases the capacity for thermotolerance, which increases chlorophyll *a:b* ratio [26,27]. Air temperature also influences the formation of chlorophyll as temperature regulates the synthesis of chlorophyll precursor [28]. In our study, the phytotron at 29–33 °C provided an

appropriate growth environment for sago palm seedlings. Those growing at 31 °C revealed higher uptake in macronutrients such as N, P, K, and Ca, which contribute to the maximum leaf area [9]. Therefore, it appears the higher uptake of nutrients induced the higher formation of leaf chlorophyll leading to greater capacity to harvest light energy. In addition, although no measurement in leaf emergence rate undertaken, we noticed that most plants showed leaf emergence at the higher air temperature, the same occurrence had previously been found in the study of sago palm seedlings' response to various ranges of air temperature. It was found that at 35 °C leaf emergence rate increased although shoot elongation rate, leaf area and root growth rate decreased. However, at 23 °C leaf emergence rate decreased along with increased root growth rate [9].

In general, sago palms reach light saturation point from 600–750  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD, although this point may vary depending on the leaf age and shaded conditions [6,29]. According to our finding, air temperature is also one of the factors affecting the light saturation point of sago palm seedlings, especially in the lower air temperature range tested. The optimum irradiances ( $\text{PPFD}_{\text{opt}}$ ) of sago palm seedlings was rather low at 25–29 °C followed by early reduction in electron transport rate as photo inhibition might have occurred due to excess light energy. This can be seen from the down-ward trend which occurred when the light intensity increased above 600  $\mu\text{mol}$  (Figure 3B). The sago palm seedlings grown at 29–33 °C maintained higher performance in light energy utilization for electron transport than those at 25–29 °C (Figure 2B). The increase in leaf temperature as long as it does not exceed the upper thermal limit, may enhances photon flux density which consequently affects the adjustment of thermotolerance in PSII and results in optimum photosynthetic rate [27,30,31]. In our study, sago palm photosynthetic optimum irradiance was higher when the plants were growing at the higher air temperature. The reduction in electron transport rate due to photo inhibition occurred when PPFD increased above 800  $\mu\text{mol}$  (Figure 2B). However, the higher maximum electron transport rate showed not significant higher between the treatments.



**Figure 3.** The leaf greenness of sago palm seedlings grown at 25–29 °C (A); and 29–33 °C (B) room temperature.

Although the sago seedlings grown at higher temperature performed higher optimum irradiance, the utilization of light energy for photosynthetic activity tends to be less efficient. High dissipation of light energy in non-photochemical quenching (NPQ) at 29–33 °C also supports this analysis. The process is a plant mechanism to protect the photosynthetic apparatus from photo damage due to excess light energy [32]. Non-photochemical quenching dissipates the excess of light energy as heat.

## 5. Conclusions

According to the above findings, we conclude that sago palm photosynthetic performance is affected by changes in ambient air temperature especially at the minimum air temperature tested.



This refers to the lower  $P_N$  performance at air temperatures ranging from 25–29 °C as compared to those at 29–33 °C. Low  $P_N$  was brought about by low values in other supporting variables such as stomatal conductance, leaf transpiration rate, maximum rubisco rate in  $CO_2$  reduction, and optimum irradiance. A low level of formation of photosynthetic pigment also becomes a limiting factor in photosynthetic rate at the lower temperatures tested. Consequently, the harvesting and utilization of light energy for photosynthetic activities was affected. From this, it could be concluded that the optimum air temperature would be associated with higher sago palm yield. Further investigation of photosynthetic response to different air temperatures using fully grown sago palms is needed to ascertain whether they show the same photosynthetic performance as in the seedling stage. If such study is able to confirm the broader representativeness of the present study, the findings might be used to make important decisions regarding the location of sago palm cultivation areas in the future for optimal production.

**Acknowledgments:** This study was supported by the Indonesia Endowment Fund for Education under the auspices of the Ministry of Finance of the Republic of Indonesia, Global-Scale Issue International Research Network Project launched by Secretariat of Agriculture, Forestry and Fisheries Research Council, MAFF of Japan, and JSPS KAKENHI Grant Numbers JP15H05245, to whom we express our gratitude.

**Author Contributions:** A.A. and H.E. conceived and designed the experiments; A.A. performed the experiments; A.A. analyzed the data; D.M. and H.E. contributed materials and tools; A.A. wrote the paper; and H.N. contributed in giving input for this paper. The interactions of the listed authors represented a true collaborative effort in this publication.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Larcher, W. *Physiological Plant Ecology*; Springer: Berlin/Heidelberg, Germany, 2003; p. 504.
2. Ehara, H. Geographical distribution and specification of *Metroxylon* palms. *Jpn. J. Trop. Agric.* **2005**, *50*, 229–233.
3. Ehara, H.; Susanto, S.; Mizota, C.; Shohei, H.; Tadashi, M. Sago palm (*Metroxylon sagu*, Areaceae) production in the eastern archipelago of Indonesia: Variation in morphological characteristics and pith-dry matter yield. *Econ. Bot.* **2000**, *54*, 197–206. [[CrossRef](#)]
4. Flach, M.; Braber, K.D.; Frederix, M.J.J. Temperature and relative humidity requirement of young sago palm seedlings. In *Proceedings of the Third International Sago Symposium*; Yamada, N., Kainuma, K., Eds.; The Sago Palm Research Fund: Tokyo, Japan, 1986; pp. 139–143.
5. Schuiling, D.L. Growth and Development of True Sago Palm (*Metroxylon sagu* Rottboll) with Special Reference to Accumulate of Starch in the Trunk, a Study on Morphology, Genetic Variation and Ecophysiology and Their Implications for Cultivation. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2009; pp. 3–9.
6. Okazaki, M.; Kimura, S.D. Ecology of the sago palm. In *The Sago Palm: The Food and Environmental Challenges of the 21st Century*; The Society of Sago Palm Studies, Ed.; Kyoto University Press: Kyoto, Japan, 2015; pp. 41–60.
7. Yamori, W.; Caemmerer, S.V. Effect of rubisco activase deficiency on the temperature response of  $CO_2$  assimilation rate and rubisco activation state: Insights from transgenic tobacco with reduced amount of rubisco activase. *Plant Physiol.* **2009**, *151*, 2072–2082. [[CrossRef](#)] [[PubMed](#)]
8. Paredes, M.; Quiles, M.J. The effect of cold stress on photosynthesis in hibiscus plant. *PLoS ONE* **2015**. [[CrossRef](#)] [[PubMed](#)]
9. Irawan, A.F.; Yamamoto, Y.; Miyazaki, A.; Tetsushi, Y. Effect of various ranges of controlled air temperatures on the early growth of sago palm (*Metroxylon sagu* Rottb.) seedlings. *Trop. Agric. Dev.* **2011**, *55*, 68–74.
10. Ehara, H.; Komada, C.; Morita, O. Germination characteristics of sago palm seeds and spine emergence in seedlings produced from spineless palm seeds. *Principes* **1998**, *42*, 212–217.
11. Mathur, S.; Agrawal, D.; Jajoo, A. Photosynthesis: Response to high temperature stress. *J. Photochem. Photobiol. B* **2014**, *137*, 116–126. [[CrossRef](#)] [[PubMed](#)]
12. Hew, C.S.; Krotkov, G.; David, T. Effects of temperature on photosynthesis and  $CO_2$  evolution in light and darkness by green leaves. *Plant Physiol.* **1968**, *44*, 671–677. [[CrossRef](#)]

13. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *345*, 659–668. [[CrossRef](#)]
14. Farquhar, G.D.; Caemmerer, V.S.; Berry, J.A. A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C3 species. *Planta* **1980**, *149*, 78–90. [[CrossRef](#)] [[PubMed](#)]
15. Sharkey, T.D.; Bernacchi, C.J.; Farquhar, G.D.; Singaas, E.L. Fitting photosynthetic carbon response curves for C3 leaves. *Plant Cell Environ.* **2007**, *30*, 1035–1040. [[CrossRef](#)] [[PubMed](#)]
16. Sharkey, T.D. What gas exchange data can tell us about photosynthesis. *Plant Cell Environ.* **2016**, *39*, 1161–1163. [[CrossRef](#)] [[PubMed](#)]
17. Klughammer, C.; Schreiber, U. Complementary PSII quantum yields calculated from simple fluorescence parameters measured by pam fluorometry and the saturation pulse method. *PAM Appl. Notes* **2008**, *1*, 27–35.
18. Björkman, O.; Demmig, B. Photon yield of O<sub>2</sub> evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* **1987**, *170*, 489–504. [[CrossRef](#)] [[PubMed](#)]
19. Ritchie, R.J.; Bunthawin, S. The use of pulse amplitude modulation (PAM) fluorometry to measure photosynthesis in a CAM orchid, dendrobium SPP. (D. CV. Viravuth pink). *Int. J. Plant Sci.* **2010**, *171*, 575–585. [[CrossRef](#)]
20. Apichatmeta, K.; Sudsiri, C.J.; Ritchie, R.J. Photosynthesis of oil palm (*Elaeis guineensis*). *Sci. Hortic. Amst.* **2017**, *214*, 34–40. [[CrossRef](#)]
21. Arnon, D.I. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1–15. [[CrossRef](#)] [[PubMed](#)]
22. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic membranes. *Methods Enzymol.* **1987**, *148*, 350–382.
23. Taiz, L.; Zeiger, E. *Plant Physiology*, 5th ed.; Sinauer Associates Inc.: Sunderland, MA, USA, 2012; pp. 106–130.
24. Koyama, K.; Takemoto, S. Morning reduction of photosynthetic capacity before midday depression. *Sci. Rep.* **2014**. [[CrossRef](#)] [[PubMed](#)]
25. Cen, Y.P.; Sage, R.F. The regulation of ribulose-1,5-bisphosphate carboxylase activity in response to variation in temperature and atmospheric CO<sub>2</sub> partial pressure in sweet potato. *Plant Physiol.* **2005**, *139*, 1–12. [[CrossRef](#)] [[PubMed](#)]
26. Camejo, D.; Rodríguez, P.; Morales, M.A.; Dell’Amico, J.M.; Torrecillas, A.; Alarcón, J.J. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.* **2005**, *162*, 281–289. [[CrossRef](#)] [[PubMed](#)]
27. Wahid, A.; Gelani, S.; Ashraf, M.; Foolad, M.R. Heat tolerance in plants: An Overview. *Environ. Exp. Bot.* **2007**, *6*, 199–223. [[CrossRef](#)]
28. Beck, W.A.; Redman, R. Seasonal variation in the production of plant pigments. *Plant Physiol.* **1940**, *15*, 81–94. [[CrossRef](#)] [[PubMed](#)]
29. Uchida, N.; Kobayashi, S.; Yasuda, T.; Yamaguchi, T. Photosynthetic characteristics of sago palm, *Metroxylon rumphii* Martius. *Jpn. J. Trop. Agric.* **1990**, *34*, 176–180.
30. Salvucci, M.E.; Crafts-Brandner, S.J. Inhibition of photosynthesis by heat stress: The activation state of rubisco as a limiting factor in photosynthesis. *Physiol. Plant.* **2004**, *120*, 179–186. [[CrossRef](#)] [[PubMed](#)]
31. Marchand, F.L.; Mertens, S.; Kockelbergh, F.; Beyens, L.; Nijs, I. Performance of high arctic tundra plants improved during but deteriorated after exposure to a simulated extreme temperature event. *Glob. Chang. Biol.* **2005**, *11*, 2078–2089. [[CrossRef](#)]
32. Ruban, A.V.; Horton, P. Spectroscopy of non-photochemical and photochemical quenching of chlorophyll fluorescence in leaves; evidence for a role of the light harvesting complex of photosystem II in the regulation of energy dissipation. *Photosynth. Res.* **1994**, *40*, 181–190. [[CrossRef](#)] [[PubMed](#)]

