

1 Article

2 **Photosynthesis of Sago Palm (*Metroxylon sagu* Rottb.)** 3 **Seedling at Different Air Temperatures**

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13 **Abstract:** Photosynthetic activities of the sago palm (*Metroxylon sagu* Rottb.) were studied to find out its
14 sensitivity to changes in ambient air temperature. The minimum ambient air temperature designed for the
15 experiment was 25–29°C, while the higher end was 29–33°C. Several photosynthetic parameters were studied to
16 support our analysis in sago photosynthetic activity, including diurnal leaf gas exchange, assimilation rate vs.
17 CO₂ concentration, leaf greenness, leaf chlorophyll content, and photosynthetic rate vs. irradiance. We found
18 that sago palm photosynthetic activity tends to be more sensitive to minimum than to maximum ambient air
19 temperature. The plants exposed to higher air temperatures had dark green leaf color associated with higher rates
20 of diurnal photosynthesis, chlorophyll content, and rubisco limited photosynthetic activity. They also exhibited
21 higher trend in optimum irradiance absorption level. Consequently, maximum light energy dissipation occurred
22 at higher temperatures.

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

24

25 **1. Introduction**

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

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

40 The optimum air temperature range for sago palm is very narrow, reported as from 25°C to 29°C [4].
41 Elsewhere it has been reported that the best growing conditions required a minimum of at least 26°C [5]. The
42 minimum temperature has been cited as an important factor limiting sago palm performance [6], which is likely
43 due to the tendency of the photosynthesis enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) to
44 be very sensitive to air temperature. Minimum temperature reduces rubisco activity, which consequently reduces
45 the utilization of RuBP by rubisco. Rubisco activase content is suppressed by up to 80% at 25°C and below [7].
46 A study on hibiscus plants reported that the effective quantum yield of PSII (Φ_{PSII}) is suppressed below 10°C
47 [8].

48 Regarding maximum temperature, morphological observation of sago palm seedlings reported that at 35°C
49 leaves expanded but become less green [9]. Higher air temperature also plays an important role in sago seed
50 germination. At 30°C, seed germination was about 20% higher than at 25°C [10]. In physiological studies,
51 moderate heat stress affects light energy harvesting as more light is dissipated for non-photochemical quenching
52 rather than photochemical quenching. Therefore, the CO₂ fixation process is depressed [11]. Another study
53 confirmed that carboxylation efficiency decreased at 39°C followed by a reduction in photosynthetic efficiency
54 [12].

55 While it has been considered that variations of ambient air temperature in the sago palm habitat might affect
56 its physiological performance, especially its photosynthetic activity, this has not been definitively proven. This

57 study aimed to provide useful data on sago palm physiology at different air temperatures beyond the little that
 58 has so far been published. Understanding the regulation of photosynthesis and chlorophyll fluorescence is very
 59 important as a tool in characterising plant reactions under abiotic stress, such as high and low temperature stress
 60 [13]. Thereby, the area for sago palm cultivation can be effectively selected to meet the need for appropriate air
 61 temperature, allowing optimum plant growth to be achieved and producing optimal yields.

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

65

66 2. Materials and methods

67

68 2.1. Plant material and culture conditions

69 The experiment was conducted in two phytotrons (glass house) with air temperatures ranging from 25–
 70 29°C and 29–33°C respectively at Nagoya University, Japan, from January to March 2017. These air
 71 temperatures were considered as the range of ambient temperature in sago palm habitats associated with tropical
 72 rainforest climate. Air relative humidity ranged from 30–50% and irradiance flux density from 600–800 μmol
 73 $\text{m}^{-2} \text{s}^{-1}$. Six one-year-old sago palm seedlings with six fully-developed leaves, grown individually in 1/10000a
 74 Wagner pots (diameter 115 mm and height 184 mm), were tested. The plant materials were obtained in seed
 75 form from Sentani District, Jayapura, Indonesia. Vermiculite was applied as the growing media for each plant.
 76 The nutrients were supplied through the application of Kimura B culture solution. The second youngest leaves
 77 were selected for all measurements. At the beginning, all plants were placed in the same phytotron with air
 78 temperature set at 25–29°C. After that six plants were moved to a phytotron with air temperature at 29–33°C.
 79 After one month of acclimation, measurement was conducted.

80 2.2. Diurnal leaf gas exchange

81 Diurnal leaf gas exchange was measured hourly from 7:00 AM to 05:00 PM. The diurnal leaf gas exchange
 82 measurement was ended at 03:00 PM to the plants grown at 25–29°C as the photosynthetic value was zero after
 83 03:00 PM. The portable photosynthesis system, Li-6400XT (LiCor Inc., USA) with 6 cm^2 leaf chamber was
 84 utilized during the measurement. The CO_2 concentration was controlled at 400 μmol and photosynthetic photon
 85 flux density (PPFD) was set at 750 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$. The CO_2 mixer was adjusted at 500 μmol and relative humidity
 86 in the leaf chamber was controlled at 40% in the phytotron. During measurement, leaf temperature was set at
 87 25°C. Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (T_r), and intercellular CO_2
 88 concentration (C_i) parameters were obtained from this measurement.

89 2.3. Assimilation rate vs. CO_2 concentration (A/C_i curve)

90

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

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

101

$$102 \quad A = V_{\text{cmax}} \left[\frac{C_c - I^*}{C_c + K_c(1 + O/K_o)} \right] - R_D \quad [1]$$

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

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

108

$$109 \quad A = J \frac{C_c - I^*}{4 C_c + 8 I^*} - R_d \quad [2]$$

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

112 2.4. Photosynthetic rate vs. Irradiance

113
 114 A light response curve was constructed using a photosynthesis yield analyzer (MINI-PAM, Walz-Germany)
 115 after dark adaptation for 20 minutes. Nine irradiance levels were given from zero to 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and each
 116 irradiance had an interval of 10 seconds to reach to steady state level. Three sago seedlings were chosen as
 117 replicates. Three leaflets from the second younger leaf of each plant were measured to obtain the mean value
 118 of each replicate.

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

$$123 \Phi_{\text{PSII}} = \frac{F_m' - F}{F_m'} = \frac{\Delta F}{F} \quad [3]$$

124
 125 F_m' is the maximum fluorescence yield in light adapted sample where all PSII is the open stage. F is yield
 126 fluorescence measured briefly before saturation pulse application, and ΔF is the increase of fluorescence
 127 induced by a saturation pulse [17]. The equation used to fit Φ_{PSII} is simple exponential decay function of the
 128 form $\Phi_{\text{PSII}} = e^{-x}$ after appropriate scaling,

$$129 \Phi_{\text{PSII}} = \Phi_{\text{PSII max}} \times e^{-k_y \text{PPFD}} \quad [4]$$

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

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

$$133 \text{ETR} = \Phi_{\text{PSII}} \times \text{PPFD} \times 0.5 \times 0.84 \quad [5]$$

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

$$137 \text{ETR} = \frac{\text{ETR}_{\text{max}} \times \text{PPFD}}{\text{PPFD}_{\text{opt}}} \times e^{1-\text{PPFD}/\text{PPFD}_{\text{opt}}} \quad [6]$$

139 The excel routine for fitting Waiting-in-Line curves was utilized to fit the ETR vs. several levels of irradiance
 140 [20]. The excel routine was obtained personally from Ritchie. The non-photochemical quenching (NPQ)
 141 parameter corresponds to the loss of potential energy which is dissipated as heat also referred to as
 142 thermodynamic loss. NPQ is calculated as:

$$143 \text{NPQ} = \frac{Y(\text{NPQ})}{Y(\text{NO})} = \frac{F_m - F_m'}{F} \quad [7]$$

144 qN is calculated as:

$$145 \text{qN} = \frac{F_m - F_m'}{F_m - F_0} \quad [8]$$

146
 147 F is yield fluorescence measured briefly before saturation pulse application, F_m is the maximum fluorescence of
 148 dark adapted leaf, F_0 is the minimum fluorescence, and F_m' is the maximum fluorescence measured at saturation
 149 pulse. The equation used to fit qN and NPQ vs. irradiance curves is simple exponential saturation functions:

$$150 \text{qN} = \text{qN}_{\text{max}} [1 - \exp(-K_{\text{qN}} \times \text{PPFD})] \quad [9]$$

151 while NPQ was calculated as:

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

154 PPF is photon flux density, K_{qN} and K_{NPQ} are exponential constants, qN_{\max} is the asymptotic maxima for qN ,
 155 and NPQ_{\max} is the asymptotic maxima for NPQ.

156 2.5. Chlorophyll content

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

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

166 2.6. Statistical analysis

168 This experiment employed completely randomized design with two ranges of air temperature, 25–29°C
 169 and 29–33°C, with three replicates. The second upper- most leaf from each replicate was used for measurement.
 170 Each datum is presented as mean \pm SE. Statistical differences were tested by student *t*-test.

172 3. Results

174 3.1. Diurnal leaf gas exchange

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

182 Stomatal conductance (g_s) and leaf transpiration rate (T_r) showed the same trend with P_N . At 25–29°C, g_s
 183 showed a slight upward trend during the observation. Low stomatal aperture at 25–29°C caused a reduction in T_r
 184 (Fig. 1B,D). At 29–33°C, the higher P_N trend was followed by higher g_s and T_r . However, intercellular CO_2
 185 concentration (C_i) at higher temperature showed a lower rate only in the first two hours of measurement (7:00 to
 186 8:00 h) and the last two hours of measurement. They exhibited almost the same trend in C_i rate between 9:00
 187 and 13:00 h (Figure 1C).

189 3.2. Photosynthetic activity and chlorophyll content

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

198

199 **Table 1.** Photosynthetic parameters, and chlorophyll content of sago palm seedlings at different air temperatures.
 200 *: significant ($p \leq 0.05$), **: significant ($p \leq 0.01$), ns: not significant ($p > 0.05$), respectively using Student *t*-test.
 201 Means \pm SE, $n = 3$.
 202

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

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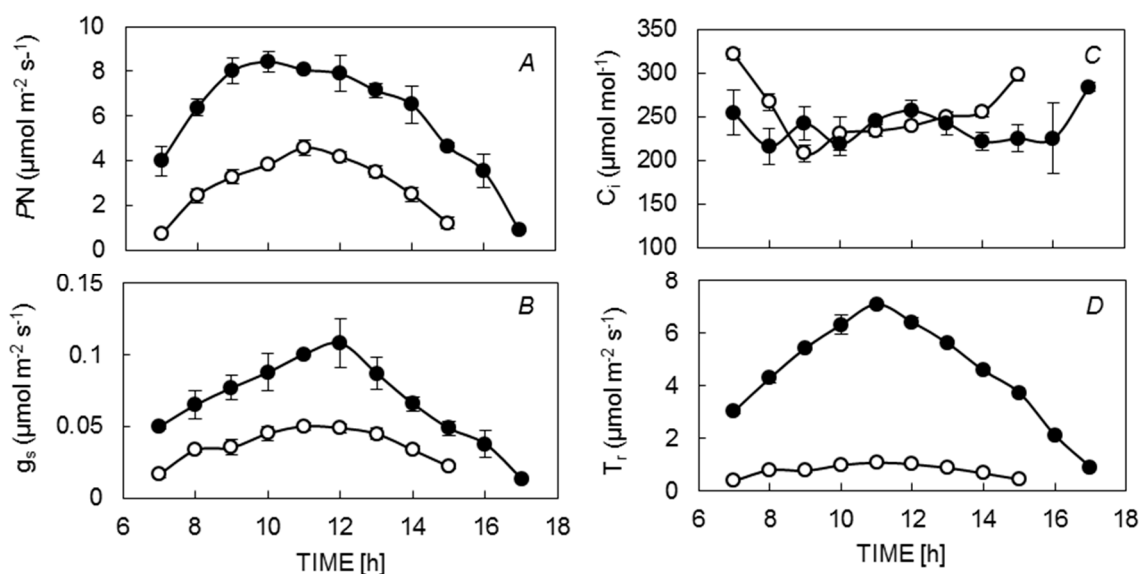
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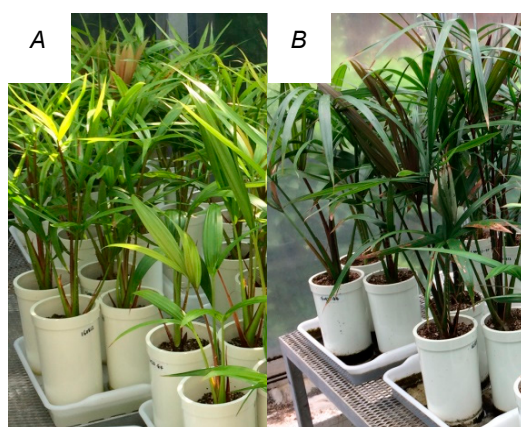


Figure 2. The leaf greenness of sago palm seedlings grown at 25–29°C [A], and 29–33°C [B] room temperature.

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

243

244

3.3. Photosynthetic rate vs. Irradiances

245 Photosynthetic rate vs. Irradiances was measured and fit using non-linear least square fit. The graphics of
 246 quantum yield of PSII at both air temperatures were obtained (Fig. 3A). The curve of quantum yield of PSII
 247 (Φ_{PSII}) vs. irradiance at both air temperatures provided a maximum effective quantum yield ($\Phi_{\text{PSII max}}$) value at
 248 25–29°C and 29–33°C. The Waiting-in-Line equation was used to fit ETR values (Fig. 3B) for both air
 249 temperature ranges tested. The variables obtained PPFD_{opt}, ETR_{max} and α_0 are shown in Table 2. The fitted qN
 250 and NPQ data (Fig 3C,D) provide information about qN_{max} and NPQ_{max}. Most of variables in Table 2 are
 251 dominated by the sago seedlings growing at 29–33°C air temperature. However, according to statistical analysis,
 252 there are no significant differences in maximum efficiency quantum yield ($\Phi_{\text{PSII max}}$), maximum electron
 253 transport rate (ETR_{max}), asymptotic photosynthetic efficiency (α_0), and maximum coefficient of non-
 254 photochemical quenching (qN_{max}) between the two air temperature ranges.

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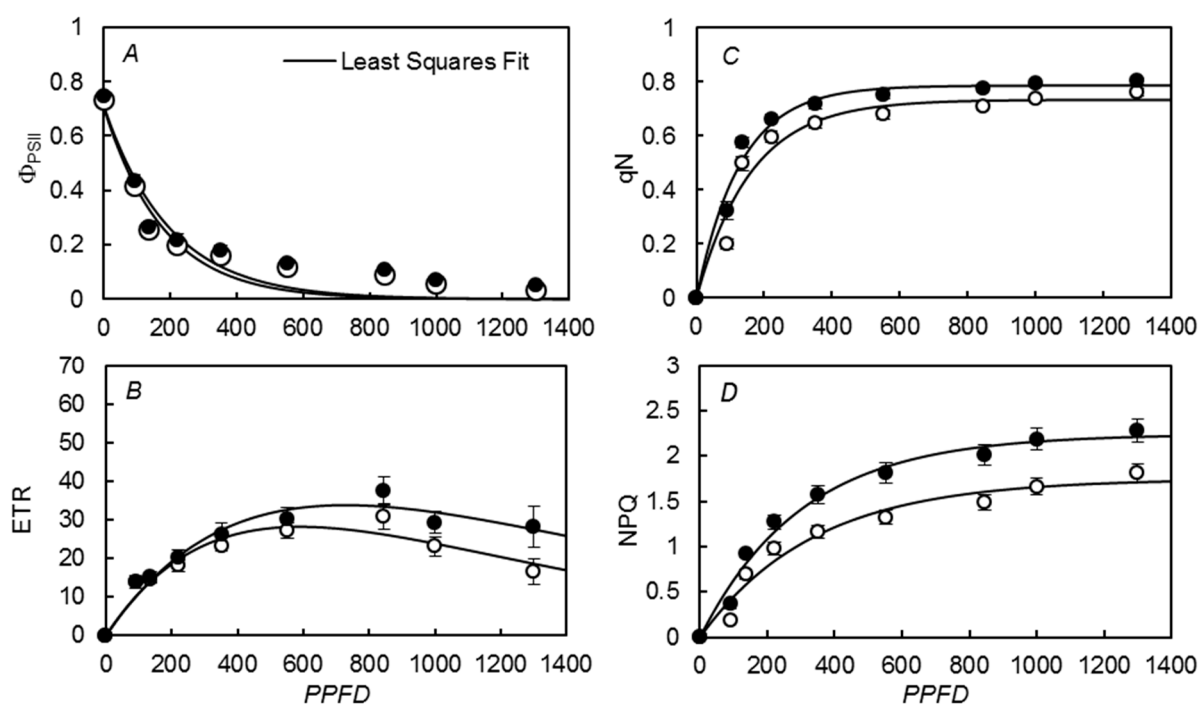
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269 **Figure 3.** (A) Photosynthetic yield (Φ_{PSII}), (B) electron transport rate (ETR), (C) coefficient non-photochemical
 270 quenching (qN), and (D) non-photochemical quenching (NPQ) vs. irradiance of sago palm seedlings (estimated
 271 via non-linear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

272

273 **Table 2.** Photosynthetic parameters (fitted by Waiting-in-Line equation) of sago palm seedling at 25–29°C and
 274 29–33°C air temperatures (Means \pm SE, $n = 3$ plants, 27 data points). *: significant ($p \leq 0.05$), ns: not significance
 275 ($P > 0.05$) as the results of Student *t*-test.

Parameters	25–29°C	29–33°C
Maximum yield ($\Phi_{\text{PSII max}}$)	0.70 \pm 0.013	0.70 \pm 0.025 ^{ns}
Optimum PPFD (PPFD _{opt})	587.00 \pm 29.81	725.59 \pm 33.32 [*]
Maximum electron transport rate (ETR _{max})	28.28 \pm 2.53	33.91 \pm 3.76 ^{ns}
Asymptotic Photosynthetic efficiency (α_0)	0.13 \pm 0.006	0.13 \pm 0.009 ^{ns}
qN _{max}	0.73 \pm 0.015	0.79 \pm 0.014 ^{ns}
NPQ _{max}	1.75 \pm 0.08	2.25 \pm 0.13 [*]

276

277 **4. Discussion**

278 At the beginning of measurement (7:00 h) sago palm seedlings revealed low photosynthetic rates in both
279 treatments. This might be due to the light not reaching sufficient levels for optimum stomatal aperture as blue
280 light induces the aperture of stomata [23]. Moreover, air temperature at that time has not reached the optimum
281 level for higher rubisco activity. Consequently, with an increase in light intensity and air temperature from
282 8:00–11:00 h, P_N , g_s , and T_r trended upwards in both treatments. However, the plants showed midday depression
283 as photosynthetic rate reduced from 12:00. This might be caused by stomatal and other non-stomatal limitations
284 such as photoinhibition, photorespiration and reduction of rubisco activity under high temperature [24]. This is
285 consistent with most field work cases, where it is difficult to obtain optimum P_N rate in sago palm when the
286 measurement is conducted after 12:00 h. This information was confirmed by our findings from diurnal leaf gas
287 exchange data. In addition, our data suggests that lower air temperature inhibited the seedlings' capacity to
288 maintain a longer P_N rate. Higher temperatures (29–33°C) appear to induce higher rubisco activity in sago palm
289 seedlings than that achieved at lower temperatures (25–29°C) (Table 1). Although producing the same diurnal
290 leaf gas exchange trend across both air temperature ranges, the sago seedlings growing at 29–33°C room
291 temperature showed higher photosynthetic rate.

292 According to the data from figure 1A, a lower net photosynthetic rate at 25–29°C was followed by lower
293 stomatal conductance results in lower leaf transpiration rate. Low transpiration rate is considered to be one of
294 the factors causing low P_N rate as CO₂ can only enter leaves through gas diffusion [23]. However, the
295 intercellular CO₂ concentration (C_i) did not show higher rate at 25–29°C. C_i revealed almost the same trend in
296 both air temperature ranges, except in the first two hours of measurement and the last two hours of measurement
297 (Fig. 1C). When plants performed higher assimilation rates, intercellular CO₂ value should show a lower rate as
298 the CO₂ is utilized during photosynthesis activity. Therefore, we assume that at 25–29°C, the photosynthetic
299 activity was not only limited by those components but also the other components such as rubisco activity, leaf
300 chlorophyll content and the light harvesting system.

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

305 The higher performance in net photosynthetic rate of sago palm seedlings at higher temperatures tested
306 also could not be separated from the support of higher photosynthetic apparatus formation, such as leaf
307 chlorophyll. The higher temperatures induced higher formation of leaf pigments such as Chl *a*, Chl *b* and
308 carotenoid. The sago seedlings grown at higher air temperatures produced leaves with a dark green color, while
309 sago seedlings grown at lower air temperature produced light green leaves. An appropriate air temperature
310 increases the capacity for thermotolerance which increases chlorophyll a:b ratio [26,27]. Air temperature also
311 influences the formation of chlorophyll as temperature regulates the synthesis of chlorophyll precursor [28]. In
312 our study, the phytotron at 29–33°C provided an appropriate growth environment for sago palm seedlings. Those
313 growing at 31°C revealed higher uptake in macronutrients such as N, P, K, and Ca, which contribute to the
314 maximum leaf area [9]. Therefore, it appears the higher uptake of nutrients induced the higher formation of leaf
315 chlorophyll leading to greater capacity to harvest light energy. In addition, although no measurement in leaf
316 emergence rate undertaken, we noticed that most plants showed leaf emergence at the higher air temperature,
317 the same occurrence had previously been found in the study of sago palm seedlings' response to various ranges
318 of air temperature. It was found that at 35°C leaf emergence rate increased although shoot elongation rate, leaf
319 area and root growth rate decreased. However, at 23°C leaf emergence rate decreased along with increased root
320 growth rate [9].

321 In general, sago palm reach light saturation point from 600–750 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, although this point
322 may vary depending on the leaf age and shaded conditions [29,6]. According to our finding, air temperature is
323 also one of the factors affecting the light saturation point of sago palm seedlings, especially in the lower air
324 temperature range tested. The optimum irradiances (PPFD_{opt}) of sago palm seedlings was rather low at 25–29°C
325 followed by early reduction in electron transport rate as photo inhibition might have occurred due to excess light
326 energy. This can be seen from the down-ward trend which occurred when the light intensity increased above
327 600 μmol (Figure 3B). The sago palm seedlings grown at 29–33°C maintained higher performance in light
328 energy utilization for electron transport than those at 25–29°C (Figure 2B). The increase in leaf temperature as
329 long as it does not exceed the upper thermal limit, may enhances photon flux density which consequently affects
330 the adjustment of thermotolerance in PSII and results in optimum photosynthetic rate [30,31,27]. In our study,
331 sago palm photosynthetic optimum irradiance was higher when the plants were growing at the higher air
332 temperature. The reduction in electron transport rate due to photo inhibition occurred when PPFD increased
333 above 800 μmol (Fig. 3B). However, the higher maximum electron transport rate showed not significant higher
334 between the treatment.

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

340 5. Conclusion

341 According to the above findings, we conclude that sago palm photosynthetic performance is affected by
342 changes in ambient air temperature especially at the minimum air temperature tested. This refers to the lower P_N
343 performance at air temperatures ranging from 25–29°C as compared to those at 29–33°C. Low P_N was brought
344 about by low values in other supporting variables such as stomatal conductance, leaf transpiration rate,
345 maximum rubisco rate in CO₂ reduction, and optimum irradiance. A low level of formation of photosynthetic
346 pigment also becomes a limiting factor in photosynthetic rate at the lower temperatures tested. Consequently,
347 the harvesting and utilization of light energy for photosynthetic activities was affected. From this it could be
348 concluded that the optimum air temperature would be associated with higher sago palm yield. Further
349 investigation of photosynthetic response to different air temperatures using fully grown sago palms is needed to
350 ascertain whether they show the same photosynthetic performance as in the seedling stage. If such study is able
351 to confirm the broader representativeness of the present study, the findings might be used to make important
352 decisions regarding the location of sago palm cultivation areas in the future for optimal production.

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