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Measuring Instant Light-Response Curve of Chlorophyll Fluorescence in Sago Palm (*Metroxylon sagu* Rottb.) Leaves: Different Time Measurements on Dark and Light-Adapted Leaf

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ABSTRACT

This study aimed to determine the appropriate time for the chlorophyll fluorescence light curve measurement in sago palms and to avoid high values variations. The photosynthetic status of three-year-old sago palm seedlings was evaluated through the chlorophyll fluorescence measurement. The chlorophyll fluorescence light curve of measurement was evaluated under different daytime measurements. Observations on $\Delta F_m/F_m'$ and ETR vs. irradiances were conducted on sago palm leaves in the morning, midday, and afternoon with light and dark adaptation. The highest ETR_{max} value was found under a light-adapted leaf; however, the E_{opt} value could not be obtained. Morning time measurement on the dark-adapted leaf is the most appropriate method and time to get an E_{opt} value with high ETR_{max}.

Keywords: Chlorophyll fluorescence, Light-response curve, *Metroxylon sagu* Rottb.

ABSTRAK

Studi ini bertujuan untuk mengevaluasi waktu yang paling optimal untuk melakukan pengukuran kurva respon tanaman terhadap cahaya melalui metode fluoresensi klorofil pada tanaman sagu sehingga variasi data yang tinggi dapat dihindari. Performa fotosintesis bibit tanaman sagu yang berusia tiga tahun dievaluasi melalui pengukuran fluoresensi. Teknik pengukuran respon tanaman terhadap beberapa intensitas cahaya pada fluoresensi klorofil dilakukan pada beberapa interval waktu yang berbeda. Pengamatan nilai $\Delta F_m/F_m'$ and ETR daun sagu terhadap beberapa level radiasi cahaya dilakukan pada pagi, siang, dan sore hari melalui pendekatan adaptasi terang maupun adaptasi gelap pada metode fluoresensi klorofil. Dari pengukuran kurva cahaya didapatkan bahwa ETR_{max} tanaman sagu berada pada nilai tertinggi pada daun dengan adaptasi terang, akan tetapi nilai E_{opt} tidak dapat diperoleh. Pengukuran kurva cahaya fluoresensi klorofil pada pagi hari dan dengan adaptasi gelap dianggap pengukuran dengan waktu dan metode yang paling tepat untuk mendapatkan nilai E_{opt} dan dengan nilai ETR_{max} yang tinggi.

Kata kuci: Klorofil flourensasi, Kurva respon cahaya; *Metroxylon sagu*

INTRODUCTION

In the 21st century, sago palm (*Metroxylon sagu* Rottb.) is a very prospective and important plant as a significant source of human carbohydrates. The growing area for sago palm is lowland land ranging from wet and tidal soil to inundated land (Azhar et al., 2018a). Besides containing high starch, which is more than 300 kg of starch per trunk (Ehara, 2005), compared to other crops, the sago palm is more resistant of facing abiotic stress conditions such as acid soils with a pH of 3.6 (Anugoolprasert et al., 2012). The performance of sago palm under abiotic stress such as water shortage condition (Azhar et al., 2020), different air temperatures (Azhar et al., 2018b), and waterlogging (Azhar et al., 2018a)

have been evaluated through photosynthetic performance. Leaf chlorophyll fluorescence (*CF*) is an effective technique for evaluating photosynthetic inhibition due to abiotic stress conditions. However, the measurement timing may affect CF's parameter value variation. There still needs to be

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more information about the optimum time for measuring chlorophyll fluorescence, especially for sago palm species.

Chl. fluorescence (*CF*) is a rapid and non-destructive method to observe inhibition in photosystem II (PSII) in plant leaves under stress conditions. Mini PAM, Heinz Walz (Effeltrich, Germany), was utilized to analyze the plant's efficiency in absorbing and using light energy for photosynthetic activity. The Quantum efficiency of photosystem II ($\Delta F_m/F_m$') can be detected instantaneously under ambient light conditions (steady stated condition). Meanwhile, photo-inhibition due to excess light energy can be determined by measuring dark-adapted leaves' maximum quantum yield of photosystem II (F_v/F_m) (Gently et al., 1989). Other important parameters can be obtained by measuring the light-response curve of Fm/Fm' under ambient light or dark-adapted conditions, such as maximum electron transport rate (ETR_{max}) at optimum irradiance (E_{opt}).

Measurement of chl. fluorescence with PAM uses an excitation energy signal emitted as fluorescence by chl. *a* molecules. This method is applied to determine the use of light absorbed by leaves and is presently the fastest and reliable phenotyping tools for the assessment of leaf photosynthesis. (Filek et al., 2015; Gulli et al., 2015; Flood et al., 2016; Guadagno et al., 2017; Gómez et al., 2018; Jonathan et al., 2020). In many cases, chlorophyll fluorescence is a powerful technique to observe the effect of abiotic stresses on the plant photosynthesis, such as detecting photoinhibition in C3 and C4 plant species (Guidi et al., 2019), leading to inhibition in photosynthetic activity.

It has been reported that under ambient light and dark-adapted conditions, PSII and electron transport rate efficiency obtained from instant light-response curve measurement using a portable chlorophyll fluorometer varies among plant species (Rascher et al., 2000). Different daytime measurements of *CF* also may affect the data obtained from the measurement. This study was done to find out the appropriate time for instant light-response curve measurement onsago palm leaves. This study aimed to measure the *CF* data in sago palm and compare the values to find the most optimum time for measurement.

MATERIALS AND METHODS

Experimental site

This study was done in a temperature-controlled glass house (phytotron) at Nagoya University, Japan, in June 2019. Growth room air temperatures ranged from 29-33°C, with 60% relative humidity. Three-year-old sago palm seedlings (n = 12) grown in 5 L pots filled with commercial black soil were used for chlorophyll fluorescence measurements with and without dark adaptation. Observations of leaf *CF* were carried out with and without dark adaptation at three different times: morning (between 08:00 to 10:00), midday (between 12:00 to 14:00), and afternoon (between 16:00 to 18:00). The *CF* measurement were done on the 2nd uppermost leaf position of each plant.

The light response curve of chl. fluorescence

PAM photosynthetic yield analyzer (Mini-PAM, Heinz Walz, Effeltrich, Germany) was utilized to assess the fluorescence of chl. *a*. Photosystem II effective quantum yield ($\Delta F_m/F_m$') was calculated as:

$$\Delta F_m/F_m' = (F_m'-F)/F_m' \qquad (1)$$

The measurement of minimum fluorescence in the dark adaptation (F_o) was conducted using <0.15 µmol m^{-2} s^{-1} modulated light of after 30 minutes of dark adaptation. Measuring conditions were set as follows: actinic light: 55 µmol m^{-2} s^{-1}: 30 seconds, measuring light: 0.15 µmol m^{-2} s^{-1}: 3 µs and saturation pulse: >5500 µmol m^{-2} s^{-1}: 0.8 seconds. The following calculation was applied to calculate photosystem II electron transport rate (ETR):

$$ETR = \frac{\Delta Fm}{Fm'} \text{ x PPFD x } \frac{PSI}{PSII} \text{ x } \begin{matrix}\text{allocation}\\ \text{factor (0.5)}\end{matrix} \text{ x } \begin{matrix}\text{leaf}\\ \text{absorptance}\\ \text{factor (0.84)}\end{matrix} \quad (2)$$

The light curve of chlorophyll fluorescence measurement was done following Azhar et al. (2020). Under ambient light and dark-adapted conditions, $\Delta F_m/F_m$' and ETR light curves were measured utilizing the program of mini-PAM light curve, as light intensity enhanced within 4.5 minutes in nine irradiance levels (0 to 1300 µmol m^{-2} s^{-1}) following each other within 30 seconds. Internal halogen lamp of the instrument provided the source of light using fiber optics and the leaf clip holder.

Light curve fitting

$\Delta F_m/F_m$' value obtained from light curve data was fitted to an exponential model,

$$\frac{\Delta Fm}{Fm'} = \frac{\Delta Fm}{Fm'_{max} \cdot e^{-kwE}} \qquad (3)$$

where $\Delta F_m/F_m$' is the quantum yield effective PSII, $\Delta F_m/F_m'_{max}$ is the quantum yield effective at theoretical zero irradiance, k_w is a constant, and E is the irradiance. ETR were plotted as light curves and fitted to a Waiting-in-Line curve:

$$ETR = (ETR_{max}\ x\ \frac{E}{E_{opt}})\ x\ e^{1-E/Eopt} \qquad (4)$$

where ETR is the rate of electron transport as a measure of the photosynthesis light reactions , E_{opt} is the optimum light, and ETR_{max} is the maximum electron transport rate for photosynthesis (Ritchie, 2015).

RESULTS AND DISCUSSION

This study found that the maximum electron transport rate (ETRmax) value of sago palm leaf resulted from CFmeasurement in the morning. Although the highest ETR_{max} value was obtained in the light-adapted leaf, to obtain the optimum irradiance (E_{opt}) value for optimum photosynthetic rate, dark adaptation on the leaf (30 minutes) must be considered be applied on the sago palm leaf before *CF* measurement. In addition, the highest E_{opt} value was obtained in the adapted leaves during morning measurement.

In eco-physiological fluorescence measurement in the field, dark adaptation on leaf aimed to estimate severe photo-inhibition represented by F_v/F_m value (Thiele & Krause, 1994). At least 20 minutes is adequate to get a well starting point of an instant-light curve and eliminate severe photo-inhibition. Severe photo-inhibition is reversible after 20-30 minutes and is brought about by dissipation of energy via the build-up of a proton gradient of electrochemical across the membranes of thylakoid and the generation of heat in the cycle of xanthophyll (Thiele, Krause & Winter, 1998). The ΔFm/Fm' and ETR light curves of *Metroxylon sagu* leaf measured under ambient and dark-adapted conditions were compared to evaluate these phenomena. High Pearson r values support this approach of curve fitting as the value of ΔF/ Fm' depend on irradiance under ambient light conditions (steady state) and dark-adapted conditions. A steeper decline of $\Delta F_m/F_m$' curves measured in dark-adapted leaves was found compared to those in the ambient light condition in this study (Figure 1A, 1B).

A period of darkness on the leaf surface causes light and dark reactions inactivation in leaf photosynthesis. Interpretation of a biophysical and biochemical based on field data is not possible as the reactivation during light curve measurement is complicated (Rascher et al., 2000). $\Delta F_m/F_m$' of am-

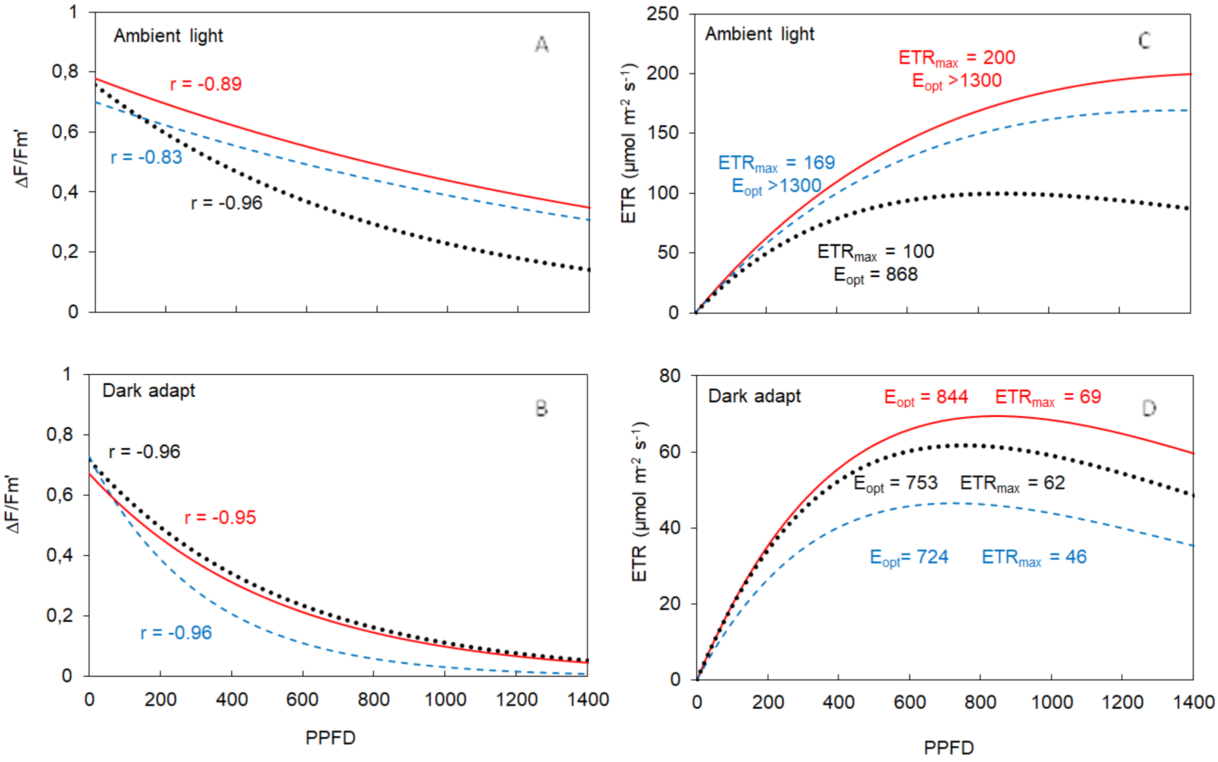

Figure 1. $\Delta F_m/F_m'$ of sago palm seedlings versus irradiances under ambient light condition (A) and dark-adapted condition (B) fitted to an exponential model. ETR versus irradiance under ambient light condition (C) and dark-adapted condition (D) fitted to a Waiting-in-Line model. Different line colors refer to the time of day when the measurement was performed: morning (red), midday (blue), and afternoon (black). Twelve curves were measured with nine irradiance levels (103 data points) for each time of measurement. PPFD (photon flux density).

bient light-adapted leaves of *Metroxylon sagu* showed similar kinetics in the morning and midday measurement, but in afternoon measurement, a steep decline of $\Delta F_m/F_m$' occurred when the irradiance intensity increased to higher levels. In dark-adapted leaves, a sharper reduction in $\Delta F_m/F_m$' was found in midday light curve measurement. It seems like in midday time, the quantum efficiency of PSII is much suppressed with higher irradiance compared to morning and afternoon measurements.

A higher value of ETR_{max} was found in ambient light conditions than in dark-adapted conditions. However, the optimum irradiance for maximum electron transport was not found in the morning and midday time measurements. Dark adaptation is needed to estimate how much the irradiance levels (E_{opt}) to get maximum electron transport. An early photo inhibition was detected in midday as the ETR value gradually decreased with the increase of light intensity (Fig. 1C, 1D). The ETR value is related to the adaptation response of plants to their environment (White & Critchley, 1999; Liu et al., 2009; Liang et al., 2010; Fu et al., 2012) and the stress response of terrestrial plants (Waldhoff et al., 2002; Li et al., 2008; Liang et al., 2010). Most research involves measuring ETR to assess photosynthetic performance, which will be related to plant biomass productivity (Huang et al., 2021).

Electrons are transported from H_2O to $NADP^+$ and temporarily stored in NADPH molecules before the reduction of CO_2. They are removed from H_2O by an Oxygen-evolving-complex (OEC). Releasing one O_2 molecule requires the oxidation of two H_2O molecules and the removal of four electrons from them (Salisbury & Ross, 1992). Through PSII, four electrons are transported in photosynthesis for each O_2 produced. Therefore, 4 µmol m^{-2} s^{-1} of ETR is equal to an approximate 1 µmol m^{-2} s^{-1} of gross photosynthesis (P_{max}) in terms of oxygen (O_2) evolution.

CONCLUSION

In conclusion, chlorophyll fluorescence traits showed a different response to daytime measurements. To avoid high variation of CF data, the measurement must be carried out simultaneously for each observation. For example, if the first measurement of CF was taken in the morning, the subsequent CF measurement must be carried out in the morning. The maximum ETR value was found in the morning measurement. Dark adaptation results in a steep reduction of $\Delta F_m/F_m$’, and the estimation value of optimum irradiance utilized for photosynthesis can be obtained.

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