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Estimation of photosynthetic activity from the electron transport rate of photosystem 2 in a film-sealed leaf of sweet potato, *Ipomoea batatas* Lam.

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Abstract

In order to evaluate the photosynthetic activity of a C_3 leaf from the electron transport rate (ETR) of photosystem 2 (PS2), a new method was devised and examined using leaves of sweet potato. In this method, both surfaces of a leaf were sealed with transparent films to stop the gas exchange between the leaf and the atmosphere; hence the functions of both photosynthetic assimilation (CO_2 uptake) and photorespiration (CO_2 release) are restricted to the inside of the leaf. After both functional rates became equally balanced, ETR of the sealed leaf (ETR_{seal}) was determined from the chlorophyll fluorescence. The measurements were conducted at different irradiances and leaf temperatures and by using leaves of different age. Under each measurement condition, ETR_{seal} showed a close positive relationship with the photosynthetic potential, or the gross photosynthetic rate measured in the air of 2 % O_2 ($P_{G2\%}$) before sealing. ETR_{seal} may become an indicator to estimate or evaluate the photosynthetic activity of C_3 leaves.

Additional key words: C_3-leaf; chlorophyll fluorescence; irradiance; leaf age; leaf-sealing method; leaf temperature; oxygen concentration; photorespiration; respiration.

Introduction

Net photosynthetic rate per unit leaf area (P_N) is usually measured by the assimilation chamber method, and its maximum value determined with a leaf having fully open stomata is regarded as a standard parameter indicating the maximum photosynthetic activity of the leaf under the measurement environmental conditions. In the measurement of this parameter, it is necessary to keep the stomata sufficiently open to minimise the stomatal resistance for gas exchange; however, we have often experienced some difficulty in the measurement, because the stomatal aperture is frequently insufficient depending on environmental conditions, and this often significantly affects P_N.

Recently, a considerable improvement has been found in the measurement technique of chlorophyll (Chl) fluorescence. This enabled more accurate knowledge on the action of photosystem 2 (PS2) in leaves. Much information is now available on the relationship between the electron transport rate in PS2 (ETR) and P_N in leaves (Genty *et al.* 1989, Harbinson *et al.* 1990, Marco *et al.* 1990, Schreiber *et al.* 1986, 1998).

In C_4 plants, P_N has a positive linear relationship with the quantum yield of PS2 or ETR over a wide range of measurement conditions. Thus the photosynthetic activity of a C_4 leaf can be predicted by measuring Chl fluorescence (Krall and Edwards 1990, Edwards and Baker 1993, Oberhuber and Edwards 1993). But such a liner relationship was not observed in C_3 plants due to the existence of photorespiration. In C_3 leaves the change in intercellular CO_2 concentration (C_i) with stomatal movement has a great effect on the rate of photorespiration (R_P), by which both P_N and ETR are affected. This may cause the loss of linearity in their relationship (Krall and Edwards 1992).

If the photosynthetic activities of C_3 leaves can be accurately and quickly estimated from ETR without measuring gas exchange rates, it should become a very useful

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Abbreviations: a – ratio (0.5) of photons distributed to photosystem 2; b – radiation absorption ratio of leaf; Chl – chlorophyll; ETR – electron transport rate at photosystem 2; ETR_{seal} – ETR of a sealed leaf; $ETR_{2\%}$ – ETR determined in air with 2 % O_2; F_m – maximum chlorophyll fluorescence; F'_m – chlorophyll fluorescence spike; F_s – chlorophyll fluorescence; F_0 – initial chlorophyll fluorescence; g_s – stomatal conductance; I – irradiance; P_G – gross photosynthetic rate; $P_{G2\%}$ – P_G determined in air with 2 % O_2; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PS – photosystem; R_D – dark respiration rate; R_P – photorespiration rate; RuBP – ribulose-1,5-bisphosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBPO – ribulose-1,5-bisphosphate oxygenase; T_c – CO_2 fixation rate; T_{cseal} – T_c of a sealed leaf; Φ_e – PS2 quantum yield.

technique for photosynthetic evaluation of C_3 leaves. For this purpose, we design here a method to estimate the photosynthetic activity of C_3 leaves from ETR measured with a film-sealed leaf, and discuss its application and theoretical background.

Materials and methods

Plants: The cultivar, Koganesengan, of sweet potato, *Ipomoea batatas* Lam., was grown in 8 000 cm^3 pots containing sufficiently fertilised sandy soil in a vinyl house set in the agricultural experimental field of Kyusyu University during the spring to the summer of 2001.

Simultaneous measurements of P_N and Chl fluorescence were done using unsealed and sealed leaves:

(*1*) **The measurements with unsealed-leaves**: At the first step, P_N, stomatal conductance (g_s), and Chl fluorescence were simultaneously measured. In the measurements, the relative humidity and the CO_2 concentration of air sent to the chamber were 50 % and 360 cm^3 m^{-3}, respectively. The O_2 concentration of air was 21 or 2 %. Leaf temperature was in the range from 18 to 34 °C, and photosynthetic photon flux density (PPFD) was from 340 to 760 μmol m^{-2} s^{-1} PPFD. P_N and g_s were measured by using an open system assimilation chamber. The leaf area set in the assimilation chamber was 6.25 cm^2 and the flow rate of air through the chamber was adjusted to 16.7 cm^3 s^{-1}. The gross photosynthetic rate (P_G) was obtained as the value of P_N *plus* the dark respiration rate (R_D). P_G determined in 2 % O_2 air was termed $P_{G2\%}$, and this parameter is the rate of photosynthesis without photorespiration, being regarded as the photosynthetic potential of a C_3 leaf.

The CO_2 concentration and vapour pressure in the reference and sample air were monitored with an infrared CO_2 analyser (*Li-6262*, *LI-COR*, USA). The concentration of O_2 was adjusted using a gas concentration controller (*GM-3A*, *KOFLOC*, Japan).

The Chl fluorescence of PS2 was monitored with a fluorescence probe (*PAM-2000*, *Walz*, Germany) equipped on the assimilation chamber. Using a leaf dark-adapted under a measuring beam (3.2 μmol m^{-2} s^{-1} PPFD, 4.8 kHz), the initial fluorescence (F_0) in the non-photosynthetic situation was measured, then the maximum fluorescence (F_m) was determined by giving a 1.2 s pulse irradiation of "saturation light" (1 800 μmol m^{-2} s^{-1} PPFD) to the leaf. After this, the time course of quenching of the fluorescence (F_s) was monitored at different irradiances and at different leaf temperatures, during which the fluorescence spike (F'_m) was periodically measured by giving pulses of "saturation light".

Based on the measurements of fluorescence, quantum yield of PS2 (Φ_e) and ETR were calculated from the equations (1) and (2), respectively.

$$\Phi_e = (F'_m - F_s)/F'_m \quad (1)$$

$$ETR = \Phi_e I\, a\, b \quad (2)$$

where I is irradiance [μmol m^{-2} s^{-1} PPFD] supplied to a leaf, and b is the ratio (b = 0.93) of leaf-absorbed photons to the incident photon. The value of b = 0.93 was calculated, as an average, from the difference between the irradiance supplied to the adaxial leaf surface and that transmitted through the leaf. Assuming the absorbed photons are distributed evenly to the two photosystems, 0.5 was used here (a = 0.5). ETR measured at 21 and 2 % O_2 airs were termed $ETR_{21\%}$ and $ETR_{2\%}$, respectively.

(*2*) **The measurements with sealed-leaves**: After the measurement of an unsealed leaf, both abaxial and adaxial surfaces of the leaf were tightly sealed with transparent films, the fluorescence of the leaf was measured in the same ranges of leaf temperatures and irradiances as mentioned above. The transparency of the film was 98 %. ETR measured with a sealed leaf was termed ETR_{seal}.

The CO_2 fixation rate (T_c) of an unsealed leaf is the summed value of P_N, R_D, and R_P, then the relationship of these parameters is written as the equation (3):

$$P_N = T_c - (R_P + R_D) \quad (3)$$

In a sealed leaf, CO_2 exchange between inside and outside of the leaf is completely restricted, *i.e.* $P_N = 0$, and hence, the functions of both CO_2 assimilation and photorespiration are forced to do within the limited place in the leaf. Accordingly, CO_2 released in photorespiration and dark respiration is retrieved as the substrate for CO_2 assimilation in a sealed leaf. When T_c of a sealed leaf is termed T_{cseal}, the Eq. (3) is rewritten as the equation (4):

$$T_{cseal} = R_P + R_D \quad (4)$$

Assuming here that the CO_2 supply from dark respiration is sufficiently small compared to that from photorespiration, T_{cseal} becomes close to the photorespiratory CO_2 release rate in a sealed leaf, as shown by the equation (5):

$$T_{cseal} \approx R_P \quad (5)$$

The CO_2 released in photorespiration is the source for CO_2 fixation in a sealed leaf to which the external CO_2 is not supplied. Hence, the rates of photorespiratory CO_2 release and photosynthetic CO_2 fixation become equal in a sealed leaf as mentioned above. T_{cseal} is estimated by the equation (6):

$$T_{cseal} = ETR_{seal}/(4 + 8) \quad (6)$$

where the number of electron equivalents to assimilate 1 CO_2 molecule is 4, and that to release 1 CO_2 by photorespiration is 8.

Results and discussion

Irradiance responses in $P_{G2\%}$, $ETR_{2\%}$, and ETR_{seal} in a young active leaf were determined at a leaf temperature of 30 °C in an irradiance range from 340 to 760 μmol m^{-2} s^{-1} PPFD. Changes in these parameters with irradiance are shown in Fig. 1*A,B*. $P_{G2\%}$ increased with an increase in irradiance, having a turning point at about 600 μmol m^{-2} s^{-1} PPFD (Fig. 1*A*). Both $ETR_{2\%}$ and ETR_{seal} in Fig. 1*B* had an almost similar irradiance response to each other, though the former was 30 to 50 % larger in parameter value than the latter.

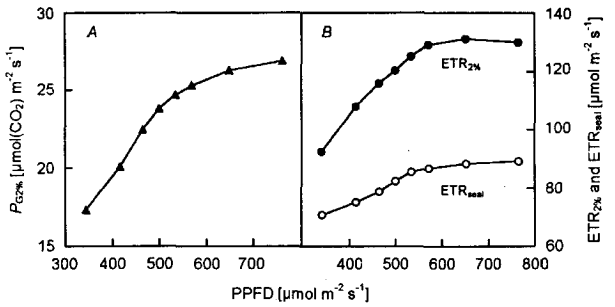

Fig. 1. Changes in $P_{G2\%}$, $ETR_{2\%}$, and ETR_{seal} with irradiance at leaf temperature of 30 °C.

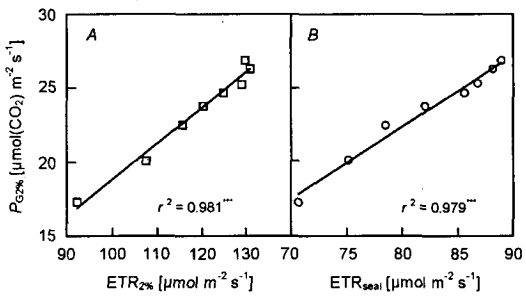

Fig. 2. Mutual relationships between $P_{G2\%}$ and $ETR_{2\%}$ or ETR_{seal}. The values are from Fig. 1. *** statistically significant at $p<0.001$.

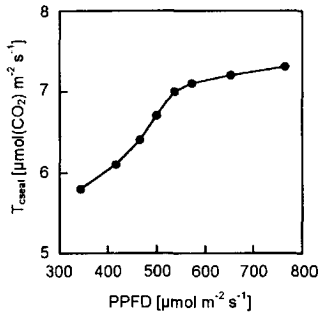

Fig. 3. Change in T_{cseal} with irradiance (PPFD) at leaf temperature of 30 °C. T_{cseal} was calculated by the Eq. (6).

Based on the values of Fig. 1, the relationships between $P_{G2\%}$ and $ETR_{2\%}$, and $P_{G2\%}$ and ETR_{seal} measured in the irradiance range from 340 to 570 μmol m^{-2} s^{-1} PPFD are shown in Fig. 2*A,B*. A highly significant linear relationship was detected between $P_{G2\%}$ and $ETR_{2\%}$ (Fig. 2*A*). P_N measured in the normal atmospheric air containing 21 % O_2 did not show a linear relationship with ETR in C_3 leaves, because the functional strength of photorespiration in leaves greatly affects the photosynthetic electron distribution (Krall *et al.* 1991). We have observed a similar fact in sweet potato leaves (Haimeirong *et al.* 2001). But when photorespiration is restricted in the air of low O_2 concentration, the sink for electrons transported from the photosystems is limited to CO_2 assimilation function, and hence, a close relationship appears between CO_2 assimilation rate and ETR (Krall and Edwards 1990, 1992). This fact is also recognised in the linear relationship in Fig. 2*A*.

Also in Fig. 2*B* there is a close relationship between ETR_{seal} and $P_{G2\%}$. This means that $P_{G2\%}$ is accurately estimated from ETR_{seal}, which can be used as an indicator estimating the photosynthetic activity of a C_3 leaf placed in different irradiances. However, the accuracy of estimated values might gradually decrease as the irradiance increases beyond this level, because energy dissipation mechanisms such as the Mehler reaction may become more activated (Oberhuber and Edwards 1993).

T_{cseal} calculated by the Eq. (6) is shown in Fig. 3. The value of T_{cseal} increased from 5.8 to 7.3 μmol(CO_2) m^{-2} s^{-1} with an increase in irradiance from 340 to 760 μmol m^{-2} s^{-1}. This parameter value is calculated under the assumption that the rates of photorespiration and assimilation in a sealed leaf are equally balanced, and R_D is not included in this balance. The R_D measured here was small (about 1 μmol m^{-2} s^{-1}), being about six to seven times lower than that of the estimated T_{cseal} of 5.8 to 7.3 μmol(CO_2) m^{-2} s^{-1}. This may suggest that the CO_2 release of dark respiration was not so strongly effective in varying the CO_2 balance in a sealed leaf, which allows us to consider that the main energy consumption in a sealed leaf is almost restricted to both CO_2 assimilation and photorespiration; hence, T_{cseal} can be estimated from Eq. 6.

Besides CO_2, ribulose-1,5-bisphosphate (RuBP) is another substrate for RuBP carboxylase/oxygenase, RuBPCO. When the external supply of CO_2 is restricted in a sealed leaf, the reproduction of RuBP might decrease with time because a part of produced photosynthates is exported out of chloroplasts. The deficit of RuBP may cause the functional reduction of CO_2 assimilation and photorespiration. The occurrence of this phenomenon should be associated with a drop of ETR_{seal}. But such a drop was not observed during the measurement time of more than 15 min. This evidence suggests that the shortage of RuBP reproduction did not occur in the sealed leaf in this time.

In addition to RuBP, O_2 is the other substrate for RuBP oxygenase (RuBPO). It is predicted that 8 electrons (e^-) and 4 e^- are required for the release of 1 molecule of CO_2 in photorespiration and in the uptake of

1 CO_2 in assimilation, respectively, in a sealed leaf. To obtain 12 electrons, 6 H_2O are oxidised to produce 3 O_2 in PS2. Since 1 CO_2 release in photorespiration requires 2 O_2 at RuBPO, then 1 O_2 is excessive, though a part of this is consumed by dark respiration. This may predict that the CO_2/O_2 balance in a sealed leaf is changed and this affects the response rate of RuBPCO and ETR_{seal}. Nevertheless, ETR_{seal} was kept constant for over 15 min as mentioned above, and in this duration we could repeat measurements of Chl fluorescence.

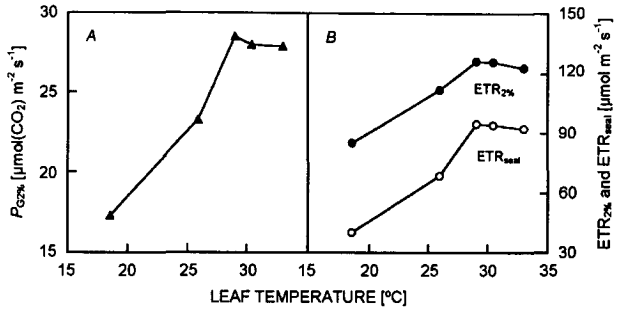

Fig.4. Changes in $P_{G2\%}$, $ETR_{2\%}$, and ETR_{seal} with leaf temperature at an irradiance of 500 μmol m^{-2} s^{-1}.

Leaf temperature is an important environmental factor significantly affecting photosynthetic parameters. The variations in $P_{G2\%}$, $ETR_{2\%}$, and ETR_{seal} with leaf temperature from 18 to 34 °C are shown in Fig. 4*A,B*. An irradiance of 500 μmol m^{-2} s^{-1} PPFD was used here. As shown in Fig. 4*A*, $P_{G2\%}$ increased with an increase in temperature and reached a highest plateau at 30 °C. A similar pattern of this was also detected in both ETR_{seal} and $ETR_{2\%}$ in Fig. 4*B*. There were positive significant linear relationships between them, that is r^2 = 0.977, r^2 = 0.978, and r^2 = 0.999 were detected for $P_{G2\%}$–$ETR_{2\%}$, $ETR_{2\%}$–ETR_{seal}, and ETR_{seal}–$P_{G2\%}$, respectively. Like this, the change in $P_{G2\%}$ with leaf temperature can be estimated from ETR_{seal}.

Next, we examined the relationship between $P_{G2\%}$ and ETR_{seal} using sweet potato leaves of different age. Fig. 5 shows the relationship between $P_{G21\%}$ and ETR_{seal} in these leaves measured in 500 μmol m^{-2} s^{-1} PPFD at a leaf temperature of 30 °C. The range of $P_{G2\%}$ was from 11.3 to 23.3 μmol(CO_2) m^{-2} s^{-1} in the leaves. The numbers in the figure were the positions of expanded leaves on a stolon. 1.5 leaves expanded per day in the summer; hence, for example, the leaf numbered 32 was about 50-d-old after expansion. As shown here, these parameters represented a linear relationship having a high statistic significance of $r^2 = 0.906^{**}$, and this fact may demon-strate that ETR_{seal} is an appropriate parameter for estima-tion of $P_{G2\%}$ in leaves of different age.

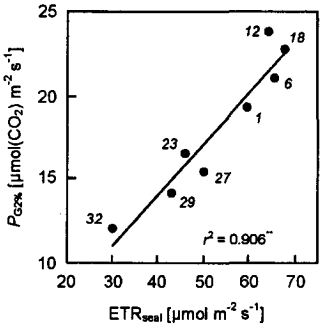

Fig. 5. The relationship between $P_{G2\%}$ and ETR_{seal} in leaves of different age. The measurements were made at a leaf temperature of 30 °C in an irradiance of 500 μmol m^{-2} s^{-1} PPFD. The numbers in the figure show the order of fully expanded leaves positioned on a stolon from young (1) to old (32). **statistically significant at $p<0.01$.

In many cases, P_N measured with a leaf having full open stomata is usually evaluated as a standard of photosynthetic activity of the leaf. One of the problems for this measurement is that a time-taking operation is often necessary to adjust and fix the action of stomata, and hence, measurements of many leaves in a limited time are difficult. Kubota *et al.* (1991) tried to peel the epidermis from a sweet potato leaf to directly measure photosynthetic rate of mesophyll with an assimilation chamber. In this method, the assimilation activity of mesophyll was determined quickly because the stomata restriction in gas exchange was removed. But the species adequate for leaf epidermis peeling are not so many, and the measurement time is restricted due to a large water loss by evaporation from the peeled leaf surface. Unlike measurements of P_N, the monitoring of Chl fluorescence is quick, and as mentioned above, ETR_{seal} shows a close relationship with $P_{G2\%}$, or the photosynthetic potential, in different measurement conditions. Hence ETR_{seal} is able to be used as one of the photosynthetic indicators for leaves of C_3 crops.

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