Evaluation of Tomato Photosynthetic Potential Based on the Chlorophyll Fluorescence of Leaflets Sealed with Transparent Film or Vaseline

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When chlorophyll fluorescence is measured on leaves with restricted gas exchange by a transparent film or Vaseline seal, the obtained electron transport rate in photosystem II (JPSII) was reported show a positive linear correlation with the maximum photosynthetic activity. This is because in a sealed leaf, the CO2 substrate for ribulose-1,5-bisphosphate-carboxylase/oxygenase is derived primarily from photorespiration. Our objective was to clarify whether the JPSII of a leaflet had a positive linear relationship with the gross photosynthetic rate at 30 mmol mol−1 oxygen. This suggests that the JPSII represents the photosynthetic carbon fixation activity. Maintaining a tight seal with the transparent film was difficult because of the gap between the film and leaflet during transpiration. In contrast, the tight seal with Vaseline enabled measurements for at least 30 min. Additionally, the measurements could be completed faster for the Vaseline-sealed leaflets. The variation in the JPSII of tomato leaflets increased with increasing leaf age. The leaf JPSII (i.e., calculated based on 10−13 leaflets) decreased with increasing leaf age. We propose that chlorophyll fluorescence measurements for Vaseline-sealed leaflets may be useful for comprehensive analyses of tomato leaf photosynthetic characteristics.

Keywords: carboxylation, electron transport, quantum yield, oxygenation, Rubisco

INTRODUCTION

When evaluating the productivity of crops, including tomato, it is important that leaf photosynthetic activities are considered. Recently, sunlight-based plant growth facilities that enable the precise control of environmental factors have been prevailing throughout Japan. Consequently, the need to optimize the control over the environmental conditions at these facilities based on plant photosynthetic potential has also increased.

Gas exchange measurements in large growth chambers (Nagaoa et al., 1984; Papadopoulos and Ormrod, 1988; Thongbai et al., 2010) and carbon isotope tracer measurements (Shishido et al., 1990; Shishido and Hori, 1991; Shishido and Kumakura, 1994; Hamamoto et al., 2000) have been commonly used for photosynthetic analyses of tomato plants. However, these measurements are only applicable for seedlings grown under ideal environmental conditions. Therefore, the development of a method to evaluate photosynthetic activities of a tomato canopy that can be used in the field or in greenhouses is necessary. Chlorophyll fluorescence measurements is a potential tool to monitor photosynthetic activities of many leaves or leaflets in a high-throughput manner as it allows rapid determination of quantum yield (ΦPSII) and the electron transport rate of photosystem II (JPSII) (Schreiber et al., 1986; Genty et al., 1989; Kooten and Snel, 1990).

In C4 plants, the net photosynthetic rate (Pn) has a positive linear relationship with ΦPSII over a wide range of intercellular CO2 concentrations (Ci). Thus, the photosynthetic activity of a Ci leaf can be predicted using a chlorophyll fluorescence measurement method (Krall and Edwards, 1990; Krall et al., 1991; Edwards and Baker, 1993). However, in C3 plants (e.g., tomato), whose photosynthetic activities are considerably influenced by photorespiration, changes in Ci due to stomatal movements affect the energy partitioning between carbon fixation and photorespiration. Thus, there is no linear relationship between ΦPSII and Pn (Krall et al., 1992). Additionally, ΦPSII and JPSII are not direct parameters of photosynthetic potential in C4 plants.

Haimeirong et al. (2002) reported that the JPSII corresponds to the photosynthetic potential of Ci sweet potato leaves sealed with transparent film to inhibit gas exchange and to equilibrate between CO2 evolution by photorespiration and O2 evolution by photosynthesis. This is based on the gas exchange occurring in a sealed leaf where the CO2 substrate for ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco) is derived primary from photorespiration. As a result, photorespiration and photosynthetic carbon assimilation could be equilibrated.

Haimeirong and Kubota (2006) also concluded that sealing leaves was easier with Vaseline than with transparent film. In addition, Vaseline-sealed leaves showed maintained its initial photosynthetic activity for a longer period of times as compared to leaves sealed with transparent films. Thus, this Vaseline-based method may be useful for...
evaluating the photosynthetic activity of tomato canopies in greenhouses or fields.

The objective of this study was to determine whether the J_{init} based on chlorophyll fluorescence measurements of sealed tomato leaves corresponds to photosynthetic activity regardless of stomatal movement. Additionally, we evaluated the utility of this method for analyzing tomato canopies during low node-order pinching and high-density cultivation.

MATERIALS AND METHODS

Plant material
Tomato (Solanum lycopersicum L.) ‘House-Momotaro’ seeds (Takii Seed Co., Ltd., Kyoto, Japan) were sown in a plastic tray filled with vermiculite. Seedlings were grown in a greenhouse until the second leaves fully expanded. The seedlings were then transplanted to No. 5 clay pots containing gravel culture and half-strength Hoagland nutrient solution. When the fifth leaves fully expanded, the Hoagland nutrient solution was replaced by half- or quarter-strength Hoagland nutrient solution. Measurement of photosynthetic activities in Vaseline-coated leaves and leaves sealed with transparent film

Chlorophyll fluorescence and P_{n} measurements for unsealed leaves and leaves sealed with transparent film

Chlorophyll fluorescence and the P_{n} were simultaneously measured for a young leaflet around the tip of the eighth leaf. During the measurements, the relative humidity and CO_{2} concentration of the growth chamber were 30% and 400 μmol mol^{-1}, respectively. To determine the maximum photosynthetic potential in the absence of photorespiration, the O_{2} concentration was adjusted to 30 mmol mol^{-1}. Leaf temperature was set at 25°C, while the photosynthetic photon flux density (PPFD) was 350, 750 and 1,750 μmol m^{-2} s^{-1}. The P_{n} was measured using the LCA4 portable photosynthesis system (Shimadzu Corp., Kyoto, Japan). The leaf area in the assimilation chamber was set at 6.25 cm², and the air flow rate through the chamber was adjusted to 270 μL L⁻¹. The gross photosynthetic rate (P_{n}) was calculated as the sum of the P_{n} and the dark respiration rate (R_{d}). The P_{n} determined at 3% O_{2} was termed the P_{n,3%}, which represents the maximum photosynthetic potential of the target leaflet.

Chlorophyll fluorescence was monitored with the PAM-2000 fluorescence probe (Heinz Walz GmbH, Effeltrich, Germany) on the assimilation chamber. The steady-state fluorescence levels (F_{s}) were measured by exposing the leaflet to different irradiances, and the maximum fluorescence level (F_{m}) was subsequently determined by treating the leaflet with a saturating flash (6,000 PPFD) for 0.8 s. Based on the fluorescence measurements, the F_{PSII} and J_{PSII} at 30 mmol mol^{-1} O_{2} (i.e., F_{PSII,3%} and J_{PSII,3%}) were calculated as follows:

\[ \Phi_{PSII} = \frac{(F_{m} - F_{s})}{F_{m}} \]
\[ J_{PSII} = \frac{L \times a \times f \times \Phi_{PSII}}{w} \]

where L refers to the PPFD incident on the leaf surface, and a is the proportion of the incident photons that was absorbed by leaves (i.e., 0.92). Assuming the absorbed photons were evenly distributed to the two photosystems, f was set to 0.5.

After analyzing the unsealed leaf, the abaxial and adaxial leaflet surfaces were tightly sealed with transparent film. Leaf fluorescence was then measured using the irradiance range mentioned above. The films were 90% transparent. The J_{PSII} value for a sealed leaf was termed J_{PSII,sealed} and was calculated as follows:

\[ J_{PSII,sealed} = L \times a \times f \times \Phi_{PSII} \]

where w is 0.9 (i.e., the film transparency).

Chlorophyll fluorescence and P_{n} measurements for unsealed leaves and leaves sealed with Vaseline

Chlorophyll fluorescence and the P_{n} were analyzed for a young leaflet around the tip of the eighth leaf. The P_{n} and R_{d} at 30 mmol mol^{-1} O_{2} were determined. Then, the abaxial and adaxial leaflet surfaces were coated with Vaseline (Nacalai Tesque, Japan) for a light transmittance of 98%. The J_{PSII,sealed} of the leaflet sealed with Vaseline was also measured.

Measurement of photosynthetic activities in Vaseline-sealed leaflets from a tomato canopy during low node-order pinching and high-density cultivation

Tomato plants were cultivated at the Mie Prefecture Agricultural Research Institute (34.6°N, 136.5°E). Tomato (S. lycopersicum L.) ‘Kanbi’ (Marutane, Japan) seeds were sown in 128-cell trays filled with fine-grain rock-wool. Seedlings were grown for 22 d under a 16-h light (25°C)/8-h dark (20°C) photoperiod with 1,200 μmol mol^{-1} CO_{2} in a closed growth chamber equipped with fluorescent light (300 μmol m^{-2} s^{-1}) (Mitsubishi Plastics Agri Dream Co., Ltd., Japan) and a recirculating system. The seedlings were transplanted into a D-tray (20 x 60 cm, 10 holes; ARMS, Japan) filled with fine-grain rock-wool, and then grown for 8 d (i.e., secondary seedlings). On 21 October 2010, the seedlings that were 25–30 cm tall were transplanted to the greenhouse. Tomato plants were pinched at the third-node-order, and the plant density was relatively high for multi-stage tomato cultivation (i.e., 5,555 plants 10 a⁻¹). Plants were treated with an Enshi standard nutrient solution, and the electrical conductivity was maintained at 1.0–1.5 dS m⁻¹ with a recirculating system.

The tomato plants in the middle of the D-tray were analyzed at the third-truss flowering stage. The oldest leaf was designated as the first leaf. Chlorophyll fluorescence was measured for the sixth, seventh, ninth, eleventh, thirteenth, and fifteenth leaves. The first, second, and third trusses were located between the eighth and ninth leaves, eleventh and twelfth leaves, and fourteenth and fifteenth leaves, respectively. The fully expanded main leaflets were numbered (i.e., leaflet 1–13; Fig. 1). Each leaflet was coated with Vaseline, after which chlorophyll fluorescence was measured three times at a PPFD of 776 μmol m⁻² s⁻¹. The J_{PSII,sealed} was calculated as described above.

RESULTS

The P_{c,U} of the leaflets from tomato plants treated with half- or quarter-strength Hoagland nutrient solution
PHOTOSYNTHESIS OF SEALED LEAF

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increased with increasing PPFDs to a maximum of 25 and 20 μmol m⁻² s⁻¹, respectively (Fig. 2). In contrast, the $P_{\text{G3%}}$ of leaflets from tomato plants treated with tap water remained unchanged at 4 μmol m⁻² s⁻¹, even with increasing PPFDs. The $J_{\text{P3%}}$ and $J_{\text{P3% seal}}$ light-response curves resembled that of $P_{\text{G3%}}$, but the $J_{\text{P3% seal}}$ values were lower than those of the $J_{\text{P3%}}$. The $P_{\text{G3%}}$ had a positive linear relationship with the $J_{\text{P3% seal}}$ and $J_{\text{P3% seal}}$ (Fig. 3).

When measurement time was longer, for example it was over 30 min, the leaflets wrapped with transparent film were not tightly sealed because of a gap between the film and leaflet surface. Therefore, we analyzed leaflets sealed with Vaseline. The $J_{\text{P3% seal}}$ light-response curves were similar for leaflets sealed with transparent film or Vaseline (data not shown). The relationship between the $P_{\text{G3%}}$ of a non-sealed leaflet and the $J_{\text{P3% seal}}$ of a Vaseline-sealed leaflet was positive and linear (Fig. 4). This indicates that the $J_{\text{P3% seal}}$ of a leaflet sealed with Vaseline may be used to represent carbon fixation activity.

Because Vaseline can form a tighter seal than transparent film, it is better able to restrict gas exchange, making it the superior sealant. Additionally, leaflets can be sealed faster with Vaseline than with transparent film. We also analyzed the temporal changes to the $J_{\text{P3% seal}}$ of Vaseline-sealed leaflets to assess the stability of the sealing treatment effects (Fig. 5). A leaflet sealed with Vaseline that had a pre-seal $J_{\text{P3% seal}}$ of 90 μmol m⁻² s⁻¹, was analyzed for 30 min. We observed that the $J_{\text{P3% seal}}$ was maintained at 60 μmol m⁻² s⁻¹. Another Vaseline-sealed leaflet with similar characteristics and pre-seal $J_{\text{P3% seal}}$ was placed in darkness for 30 min.

**Significant at $P < 0.01$.**

![Fig. 1](image1.png) Fig. 1 Numbering of tomato leaflets. The numbers were assigned to the fully expanded main leaflets from the leaf apex to the base.

![Fig. 2](image2.png) Fig. 2 Effect of photosynthetic photon flux density (PPFD) on the $P_{\text{G3%}}$ (A), $J_{\text{P3%}}$ (B) and $J_{\text{P3% seal}}$ (C) at 25°C. The $P_{\text{G3%}}$ and $J_{\text{P3%}}$ were measured at 30 mmol mol⁻¹ O₂ and 400 μmol mol⁻¹ CO₂. The plants were cultivated with a half-(represented by diamonds) and quarter- (represented by squares) strength Hoagland nutrient solution and tap water (represented by triangles). The $J_{\text{P3% seal}}$ was measured using a leaflet sealed with transparent film. Data are presented as means $\pm$ SE ($n=4$).

![Fig. 3](image3.png) Fig. 3 Relationships between the $P_{\text{G3%}}$ and $J_{\text{P3%}}$ (A) or $J_{\text{P3% seal}}$ (B). The presented values are from Fig. 2.

**Significant at $P < 0.01$.**

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min to decrease Rubisco activity. The \( J_{\text{Ftotal}} \) over the subsequent 30 min remained at 40 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).

The \( J_{\text{Ftotal}} \) values for tomato leaflets sealed with Vaseline during low node-order pinching and high-density cultivation are presented in Fig. 6. The analysis of 68 leaflets required 2.5 h. The \( J_{\text{Ftotal}} \) of the fifteenth leaf, which was the top fully expanded leaf, was relatively stable, with a low coefficient of variation (i.e., 0.126). However, the coefficient of variation for the \( J_{\text{Ftotal}} \) increased with increasing leaf age (i.e., lower leaf positions). Additionally, the \( J_{\text{Ftotal}} \) for the first to third leaflets (i.e., leaf apex) was lower than that for the middle leaflets in the seventh, ninth, and thirteenth leaves. The mean \( J_{\text{Ftotal}} \) for each leaf was relatively high among the upper leaves (i.e., eleventh to fifteenth leaves), but decreased at lower leaf positions (Fig. 6).

**DISCUSSION**

Haimeirong et al. (2002) and Haimeirong and Kubota (2006) reported that the \( J_{\text{Ftot}} \) of sweet potato plants (i.e., \( C_3 \) plants) sealed with transparent film or Vaseline might be equivalent to the carbon fixation activity (i.e., \( J_{\text{PSII}} \)). The \( J_{\text{Ftotal}} \) can be quickly measured and is relatively stable over time (Haimeirong and Kubota, 2006). Therefore, we consider that our method is appropriate for the comprehensive analysis of the photosynthetic activities of tomato plants, which produce many leaflets.

The \( J_{\text{Ftot}} \) and \( J_{\text{Ftot}} \) sealed with transparent film (i.e., \( J_{\text{Ftotal}} \)) light-response curves resembled that of \( P_{\text{G3%}} \), but the \( J_{\text{Ftotal}} \) values were lower than those of the \( J_{\text{Ftotal}} \) (Fig. 2). This could be attributed to decrease in consumption of energy from electron transport by the carboxylation in the sealed leaflet which carboxylation and oxygenation is equilibrated. The \( J_{\text{Ftotal}} \) measurements of tomato leaflets sealed with transparent film or Vaseline were positively correlated with the CO2 exchange rate at low O2 concentrations (e.g., 30 mmol mol\(^{-1}\)), which inhibit photorespiration (Figs. 3, 4). These results suggest that the \( J_{\text{Ftotal}} \) may correspond to the carbon assimilation activity in tomato leaves. However, when the measurement time was longer, a gap between the leaflet and the transparent film developed during the measurements because of leaflet transpiration. Therefore, a tight seal was formed for a relatively brief period. Similar observations were described for sweet potato (Haimeirong and Kubota, 2006). Gaps between tomato leaflets and the transparent film formed easily, likely because of the high transpiration of tomato plants. In contrast, the \( J_{\text{Ftotal}} \) measurements for Vaseline-sealed leaflets were stable for at least 30 min (Fig. 5). Thus, Vaseline may be a better sealant than transparent film for inhibiting gas exchange in tomato leaflets.

The \( J_{\text{Ftotal}} \) of a leaflet incubated in darkness for 30 min decreased by approximately 30% compared with the pre-treatment \( J_{\text{Ftotal}} \) (Fig. 5). A previous study revealed that Rubisco (i.e., CO2 fixation enzyme) is activated by light, and inactivated by 2-carboxy-D-arabinitol-1-phosphate in darkness (Parry et al., 2008). The observed decrease in the \( J_{\text{Ftotal}} \) after the incubation in darkness may have been due to the inactivation of Rubisco. Additionally, the dark-induced decrease in the \( J_{\text{Ftotal}} \) was maintained at the same level for at least 30 min, even under light. This suggests that the Vaseline seal also inhibits the light-induced activation of Rubisco activity. The photo-activation of Rubisco occurs because of the chaperone function of Rubisco activase, which is activated by ATP produced during photo-absorption (Portis Jr., 2003). In leaflets with inhibited gas exchange, there may be an insufficient amount of available ATP to activate Rubisco activase. This insufficiency of chloroplastic ATP could be related to decrease in the electron transport between PSII and PSI in the sealed leaflets as shown in Fig. 2. The sealing treatment seems to maintain Rubisco in the pre-treatment active state, implying that \( J_{\text{Ftotal}} \) may correspond to the in situ Rubisco activity.

The photosynthetic activity of tomato plants during
low node-order pinching and high-density cultivation was evaluated based on the chlorophyll fluorescence of sealed leaflets. In this study, we observed that the \( J_{\text{PSII}} \) of the main leaflets (i.e., 9–13 per leaf) was not necessarily consistent (Fig. 6). Additionally, the \( J_{\text{PSII}} \) measurements for leaflets located in the middle of leaves (i.e., leaflets 6–11) were more stable than the measurements for the leaflets at the leaf apex. In most previous studies regarding the photosynthetic rate of tomato plants, the apex leaflets were analyzed. However, our results suggest that the apex leaflets should not be used. Because our data are from only one plant, this study will need to be repeated with more replicates.

The mean leaf \( J_{\text{PSII}} \) decreased with increasing leaf age among samples collected below the second truss (Fig. 6), which is consistent with the findings of Shishido et al. (1990), who observed that the photosynthetic rate of tomato leaves decreased with increasing leaf age among samples harvested below the first truss. This suggests that the \( J_{\text{PSII}} \) based on chlorophyll fluorescence measurements of sealed leaflets may be used to represent photosynthetic activity.

In this study, we confirmed that our chlorophyll fluorescence-based analysis of sealed tomato leaflets is suitable for investigating the photosynthetic characteristics of tomato leaves. This method is faster and more convenient than other related methods. Advances in the methods available for analyzing the photosynthetic properties of tomato leaves have been limited partly because selecting an appropriate target leaf for measurements can be challenging. The method described herein may be applicable for comprehensive analyses of the photosynthetic activities of tomato leaves.

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