Photochemical Analysis for Selection of Stress Tolerant Plant

Sung Yung Yoo1, Mikyung Lee1, Suk Yong Ha1, Tae Wan Kim1*

1) Institute of Ecological Phytochemistry/Department of Plant Life and Environmental Science, Hankyong National University, 327 Jungang-ro, Anseong, Gyeonggi-do 456-749, The Republic of Korea

*) Corresponding Author: taewkim@hknu.ac.kr

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Abstract—This electronic document is a “live” template and already defined. This study was carried out to selection of abiotic stress tolerant plant species of sixty-eight plant species at Hantaek Botanical Garden in Korean using photochemical analysis. Many abiotic stresses have led to a decline in agricultural productivity and the disappearance of ecologically important plant species. Chlorophyll a fluorescence is a powerful tool to measure environmental stress response in plants. The polyphasic OJIP fluorescence transient was used to evaluate the behavior of Photosystem II (PSII) and Photosystem I (PSI) during the entire experiment period (from July to August). The photochemical reaction showed higher maximal chlorophyll fluorescence (Fp) intensity under heat and wet stress in forty-one plant species. In twenty seven plant species, chlorophyll fluorescence intensity showed a significant decrease. In wet stress, the fluorescence parameters related with electron transport on PSII and PSI reduced over twenty percent. As a result of the identifying under heat and wet stress, energy dissipation per reaction center (D1o/RC) was heavily affected. It was clearly indicated that the connectivity between photosynthetic PSII and PSI, i.e. electron transport, was far effective in the selection of stress tolerant plants.

Keywords—abiotic stress, photochemical analysis, photosystem I, photosystem II, tolerant assessment

I. INTRODUCTION

The chlorophyll a fluorescence (Chl a) analysis is to interpret how efficient the light energy absorbed by chlorophylls is used to drive photosynthesis (photochemical energy), and how excess energy is released as non-photochemical energy, such as heat and chlorophyll fluorescence. It is easily measured and has been a powerful tool to investigate the physiological stresses in plants [1].

The analysis of the kinetics of Chl a fluorescence has been a widespread non-invasive technique used extensively for the study of organic photosynthetic organisms, both in basic and applied research [2]. Chl a fluorescence is now being used by a lot of researchers as a probe for various aspects of photosynthesis from excitation energy transfer in picosecond time scale to CO₂ fixation in minutes. When photosynthetic samples, such as dark-adapted leaf or a suspension of higher plant, algal or cyanobacteria cells, are illuminated, Chl a fluorescence intensity shows characteristic changes called fluorescence induction, fluorescence transient so called the Kautsky’s effect [3].

For higher plants and algae, Chl a fluorescence induction curve measured under continuous light has a fast increasing phase less than a second, and a slow decreasing phase within few minutes duration. These polyphasic transients have commonly four inflexion points. The fast phase is labeled as OJIP, where O is for origin, the first measured minimal level, J and J are intermediate levels, and P is the peak [2,3,4].

The conventional understanding of the OJIP transient is based mainly on the hypothesis that PSII is responsible for Chl a variable fluorescence, and that the rate of PSII photochemical conversion is limited by the electron acceptor side [5]. It was further suggested that the fluorescence yield is controlled by a PSII acceptor quencher, i.e. the bound plastoquinone Qₐ in its oxidized state [6,7,8]. Also, the concentration of reduced Qₐ (Qₐ⁻), was affected by the kinetics of several different redox reactions in the photosynthetic electron transport chain. This being so, the OJIP transient has the potential to be used for the characterization of the photochemical quantum yield of PSII photochemistry, and the electron transport activity. Meanwhile, the shape of the OJIP transient has been found to be very sensitive to stress caused by changes in different environmental conditions, such as light intensity, temperature, drought, atmospheric CO₂ or ozone elevation and chemical influences, as well as by senescence [9,10,11,12,13].

II. MATERIALS AND METHODS

A. Plant materials

Sixty eight species growing at the Hantaek Botanical Gardens in the central region of Korean Peninsula were used for photochemical analysis. Photochemical analyzes were performed on the leaves of plants before and after the rainy season and in the hot season of mid-August and September.

B. Measurement of chlorophyll fluorescence

The samples were dark-adapted for 30 min before the fluorescence measurement. Samples put a clip on a leaves for darkness. Immediately after dark-adaptation for 30 minute, samples were exposed to a saturating light pulse of 3000 μmol m⁻² s⁻¹. The Chl a fluorescence emission induced by the strong
light pulses was measured and measurement of Chl a fluorescence was replicated three times. All leaves exhibit a polyphasic Chl a fluorescence rise during the first second of illumination after dark adaptation. The measured data was used for the calculation according to the JIP-test equations (2,14,15) (TABLE 1).

**TABLE 1. INFORMATION SELECTED FROM THE FAST OJIP FLUORESCENCE INDUCTION (DATA NECESSARY FOR THE CALCULATION OF THE SO-CALLED JIP PARAMETERS)**

| OJIP parameters | Formula | Description |
|-----------------|---------|-------------|
| $F_0$ | F50μs; fluorescence intensity at 50 μs | Maximal fluorescence intensity |
| $F_j$ | Fluorescence intensity at j-step (at 2 ms) | Relative variable fluorescence at the J-step |
| $F_i$ | Fluorescence intensity at i-step (at 60 ms) | Relative variable fluorescence at the I-step |
| $F_M$ | Maximal fluorescence intensity | $M_0$ or ($ΔV / Δt)_0 = TR_o / RC - ET_o / RC$ Approximate value of the initial slope of relative variable Chl fluorescence curve $V_j$ (for $F_0 = F_{50\mu s}$) |
| $V_j = (F_j - F_0)/(F_M - F_0)$ | Relative variable fluorescence at the J-step |
| $V_i = (F_i - F_0)/(F_M - F_0)$ | Relative variable fluorescence at the I-step |

Quantum yields and efficiencies/probabilities

- $\phi_{Po} = J_o / o_f = 1 - F_o / F_M$ Maximum quantum yield of primary PSII photochemistry
- $\phi_{Pt} = J_o / o_f = 1 - F_o / F_M$ Quantum yield of primary PSII photochemistry
- $\phi_{ET_o} = J_{ET_o} / o_f = 1 - F_o / F_M$ Quantum yield of the electron transport flux from QA to QB
- $\phi_{RE_o} = J_{RE_o} / o_f = 1 - F_o / F_M$ Quantum yield of the electron transport flux until the PSI acceptors
- $\psi_{ET_o} = J_{ET_o} / o_f = 1 - V_j$ Efficiency/probability with which a PSII trapped electron is transferred from QA to QB
- $\psi_{RE_o} = J_{RE_o} / o_f = 1 - V_j$ Efficiency/probability with which a PSII trapped electron is transferred until PSI acceptors

Specific energy fluxes (per active PSII reaction center)

- ABS/RC = (MO/VJ) / (1/φPt) Average absorbed photon flux per PSII reaction center (or also, apparent antenna size of an active PSII)
- $\gamma_{RC2} = ChlRC/Chltot$ Probability that a PSII Chl functions as RC
- $RC/ABS = φ_{Po} / VJ / MO = \gamma_{RC2} / (1 - \gamma_{RC2})$ Number of QA reducing RCs per PSII antenna Chl
- $TR_o/RC = MO / VJ$ Maximum trapped exciton flux per PSII
- $ET_o/RC = (MO/VJ) / (1 - VJ)$ Electron transport flux from QA to QB per PSII
- $RE_o/RC = (MO/VJ) / (1 - VI)$ Electron transport flux until PSI acceptors per PSII

Information selected from the fast OJIP fluorescence induction (data necessary for the calculation of the so-called JIP parameters)

“Performance” indices (combination of parameters)

- $PI_{ABS} = \gamma_{RC2} / (1 - \gamma_{RC2}) \cdot [\phi_{Po}/(1 - \phi_{Po}) \cdot [\psi_{ET_o}/(1 - \psi_{ET_o})]$ Performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of QB acceptors
- $PI_{ABS_total} = PI_{ABS} \cdot [\delta_{RE_o} / (1 - \delta_{RE_o})]$ Performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors

Driving forces (total driving forces for photochemical activity)

- DI_ABS_total = log(PI) Driving force on absorption basis
- DI_CS_total = log(PI) Driving force on cross section basis

**III. RESULTS AND DISCUSSION**

The chlorophyll fluorescence levels of 68 species grown under high humidity and/or high temperature conditions were analyzed. Forty one species maintained chlorophyll fluorescence levels even under high humidity conditions. Twenty seven plant species maintained chlorophyll fluorescence under high temperature conditions.

In most plants, photochemical light utilization efficiency increased in proportion to the increase in
chlorophyll fluorescence intensity (Fig. 1). I electron transfer energy fluxes (RE1o / RC) were low at 74% and 65%, respectively, and then recovered. The energy fluxes per section (Cross section, CS) in plant species such as Rhus tricocarpa Miq. and Hemerocallis coreana Nakai were enhanced in both periods, but the PSI electron transfer energy flux (RE1o / RC) under high temperature conditions was activated. The average ratio was 80% due to a decrease in the reaction center (RC / ABS). Dendranthema zawadskii var. latilobum (Maxim.) Kitam. reacted in contradiction to Rhus tricocarpa Miq., and the energy flux per RC was high, but the electron transfer energy flux per cross section (ETo / CS, RE1o / CS) was very high at 66% and 50% levels, respectively. Those were low values. Therefore, it was confirmed that the light use efficiency per leaf cross section is almost determined by the chlorophyll and the maximum light quantity (Fig. 1).

As shown in Fig. 2, PI_ABS seems to have a very high correlation with PI_total_ABS and DF_total_ABS. The values measured before the rainy season (July 1st) and after (August 14th) showed very meaningful results. Twenty-five species, including Rhus tricocarpa Miq. showed a high photochemical performance index under humid conditions, and most plants (24 species) were found to be Group I and Group III. The fifteen species such as Kirengeshoma koreana Nakai had lower PI_TOTAL_ABS under high humidity conditions than in August, and six species such as Filipendula palmata (Pall.) Maxim. had lower PI_ABS. And twenty-one species such as Asarum maculatum Nakai were found to have low PI ABS and PI TOTAL ABS in excessive humidity.

Zushi et al. [16] reported that PS II energy flux decreased under PS under heat stress conditions. In general, PS II reacts sensitively to high temperature as an unstable component, and PS I has relatively high heat resistance, so that the energy flux of PS II decreases under high temperature conditions [17, 18]. The PI_total_ABS value which means the activity of electron transfer from PSII to PSI, was found to vary greatly depending on the plant species.

| No. | Scientific Name                      | Wet | High Temp. | Group |
|-----|-------------------------------------|-----|------------|-------|
|     |                                      | (S*T*) | (S~T) | (I~IV) |
| 1   | Polygonatum humile Fisch. Ex Maxim. | S    | T          | II    |
| 2   | Rhus tricocarpa Miq.                | T    | T          | I     |
| 3   | Asarum maculatum Nakai             | T    | T          | I     |
| 4   | Hemerocallis coreana Nakai         | T    | T          | I     |
| 5   | Ligularia fischeri (Ledeb.) Turcz.  | T    | S          | III   |
| 6   | Adiantum pedatum L.                | S    | T          | II    |
| 7   | Ranunculus japonicus Thunb.         | S    | T          | II    |
| 8   | Dendranthema zawadskii var. latilobum (Maxim.) Kitam. | S    | T          | II    |
| 9   | Hanabusaya asiatica (Nakai) Nakai   | T    | T          | I     |
| 10  | Aster ageratoides Turcz.            | T    | T          | I     |
| 11  | Physalis alkekengi var. franchetti (Mast.) Makino | S    | T          | II    |
| 12  | Kirengeshoma koreana Nakai         | T    | T          | I     |
| 13  | Valeriana dageletiana Nakai ex F.Maek. | T    | T          | I     |
| 14  | Hemerocallis thunbergii Baker       | T    | T          | I     |

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Rice varieties which are highly sensitive to submergence significantly decreased the ET/CSo, RC/CSo and performance index (PI). However, resistant varieties maintained higher values of this parameter by preventing damage to both the electron donor and acceptor [19].

These results indicated that ET2o/CS, RE1o/CS, and DIo/CS were considerably differed between PS I and PS II in energy flux under wet and high temperature conditions.
Fig. 2. Relationship of performance indice for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors

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