Original Paper

Photochemical Analysis for Selection of Stress Tolerant Plant

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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 (DIo/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 oxygenic 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 CO_2 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 I 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 Q_A in its oxidized state [6,7,8]. Also, the concentration of reduced Q_A (Q_A^-), 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 CO_2 or ozone elevation and chemical influences, as well as by senescence [9,10,11,12,13].

II. Mateials 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. Measurment 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 I. INFORMATION SELECTED FROM THE FAST OJIP FLUORESCENCE INDUCTION (DATA NECESSARY FOR THE CALCULATION OF THE SO-CALLED JIP PARAMETERS)

OJIP parameters	
F_0	F50μs; fluorescence intensity at 50 μs
F_J	Fluorescence intensity at j-step (at 2 ms)
F_i	Fluorescence intensity at i-step (at 60 ms)
F_M	Maximal fluorescence intensity
$F_V = F_M - F_0$	Maximal variable fluorescence
$V_J = (F_J - F_0)/(F_M - F_0)$	Relative variable fluorescence at the J-step
$V_i = (F_i - F_0)/(F_M - F_0)$	Relative variable fluorescence at the I-step
M_0 or $(\triangle V / \triangle t)_0 = TR_0 / RC - ET_0 / RC$ $= 4\,(F_{300} - F_0)/(F_M - F_0)$	Approximate value of the initial slope of relative variable Chl fluorescence curve V_t (for $F_o = F_{50\,\mu s}$)
Quantum yields and efficiencies/probabilities	
$\varphi_{Po} \equiv J^{TR}o/J^{ABS} = 1 - F_O/F_M$	Maximum quantum yield of primary PSII photochemistry
$\varphi Pt \equiv J^{TR}o/J^{ABS} = 1 - F_t/F_M$ $= \varphi_{Po} \cdot (1 - Vt)$	Quantum yield of primary PSII photochemistry
$\varphi ET2o \equiv J^{ET2}o/J^{ABS} = 1 - F_J/F_M$ $= \varphi Po \cdot (1 - V_J)$	Quantum yield of the electron transport flux from Q_A to Q_B
$\varphi RE1o \equiv J^{RE1}o/J^{ABS} = 1 - F_I/F_M$ $= \varphi Po \cdot (1 - V_I)$	Quantum yield of the electron transport flux until the PSI electron acceptors
$\psi ET2o \equiv J^{ET2}o/J^{TR}o = 1 - V_J$	Efficiency/probability with which a PSII trapped electron is transferred from Q_A to Q_B
$\psi RE1o \equiv J^{RE1}o/J^{TR}o = 1 - V_I$	Efficiency/probability with which a PSII trapped electron is transferred until PSI acceptors
$\delta RE1o \equiv J^{RE1}o/J^{ET2}o$ $= (1 - V_I)/(1 - V_J)$	Efficiency/probability with which an electron from Q_B is transferred until PSI acceptors
Specific energy fluxes (per active PSII reaction center)	
$ABS/RC = (MO/VJ) \cdot (1/\varphi Po)$	Average absorbed photon flux per PSII reaction center (or also, apparent antenna size of an active PSII)
$\gamma RC2 \equiv ChlRC/Chltot$	Probability that a PSII Chl functions as RC
$RC/JABS = \varphi Po \cdot VJ/MO$ $= \gamma RC2 /(1 - \gamma RC2)$	Number of QA reducing RCs per PSII antenna Chl
$TRo/RC = MO/VJ$	Maximum trapped exciton flux per PSII
$ET2o/RC = (MO/VJ) \cdot (1 - VJ)$	Electron transport flux from QA to QB per PSII
$RE1o/RC = (MO/VJ) \cdot (1 - VI)$	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)	
$PI\_ABS = [\gamma RC2 /(1 - \gamma RC2)] \cdot [\varphi Po/(1 - \varphi Po)] \cdot [\psi ET2o/(1 - \psi ET2o)]$	Performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of QB acceptors
$PI\_ABS\_total = PI\_ABS \cdot [\delta RE1o /(1 - \delta RE1o)]$	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).
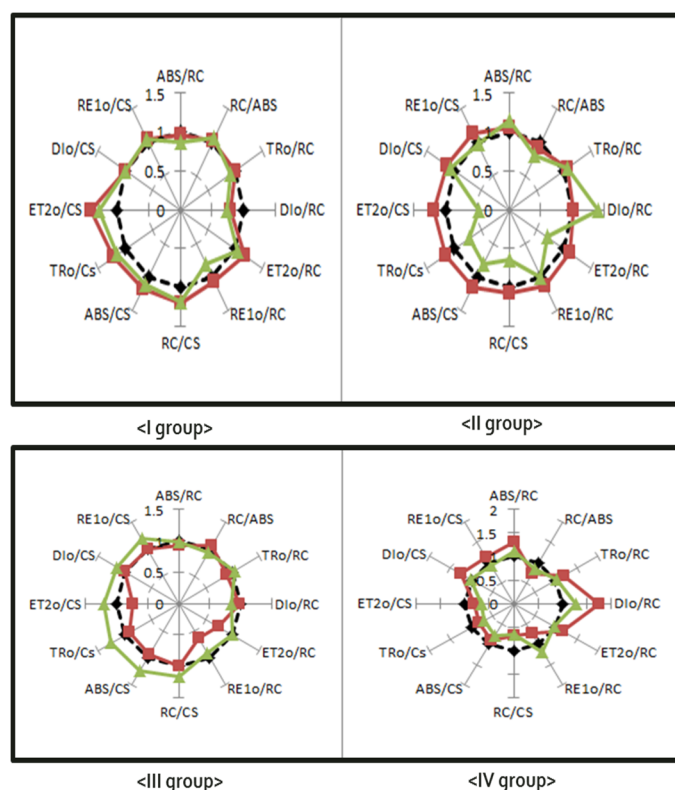

Fig. 1. Grouping of four types on the basis of photochemical parameters.

As shown in Figure 1 and Table II, plant species resistant to both high temperature and humidity belong to Group 1, and 31 species including *Rhus tricocarpa* Miq. were found. In the case of Group II, it is resistant to high temperatures, but it is a sensitive plant species that belongs to 18 species including *Ligularia fischeri* (Ledeb.) Turcz.. In addition to *Polygonatum humile* Fisch. Ex Maxim., it was classified into 15 species that are vulnerable to high temperatures and resistant to excessive humidity (Group III). In the case of Group IV, it was possible to classify the plant species as the weakest to abiotic stress such as high temperature and humidity.

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.

TABLE II.   TOLERNCE GROUPING OF 68 SPECIES ANALYSED ON THE BASIS OF PHOTOCHEMICAL PARAMETERS AND PERFORMANCE INDICE

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. franchetii (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

#	Species			
15	*Astilbe rubra* Hook.f. & Thomson	T	T	I
16	*Caryopteris divaricata* (Siebold & Zucc.) Maxim.	T	T	I
17	*Filipendula palmata* (Pall.) Maxim.	T	T	I
18	*Codonopsis lanceolata* Benth. & Hook.f. ex Trautv.	T	S	III
19	*Platycodon grandiflorum* (Jacq.) A.DC.	T	S	III
20	*Aralia cordata* var. *continentalis* (Kitag.) Y.C.Chu	T	S	III
21	*Caltha palustris* L. var. *palustris*	T	T	I
22	*Lychnis cognata* Maxim.	S	T	II
23	*Allium senescens* L.	T	S	III
24	*Polygonatum odoratum* var. *pluriflorum*	S	T	II
25	*Aristolochia manshuriensis* Kom.	S	T	II
26	*Lilium distichum* Nakai ex Kamib.	T	T	I
27	*Coreanomecon hylomeconoides* Nakai	T	S	III
28	*Liriope platyphylla* F.T.Wang & T.Tang	S	T	II
29	*Viola selkirkii* Pursh ex Goldie	T	T	I
30	*Polypodium crassifolium* L.	S	S	IV
31	*Scopolia japonica* Maxim.	T	T	I
32	*Cynanchum ascyrifolium* (Franch. & Sav.) Matsum.	S	S	IV
33	*Veratrum oxysepalum* Turcz.	S	S	IV
34	*Parasenecio firma* (Kom.) Y. L. Chen	T	T	I
35	*Iris sanguinea* Donn ex Horn	T	S	III
36	*Rhododendron dauricum* L.	T	T	I
37	*Epimedium koreanum Nakai*	T	T	I
38	*Campanula takesimana Nakai*	T	T	I
39	*Anemone stolonifera Maxim.*	T	T	I
40	*Codonopsis ussuriensis* (Rupr. & Maxim.) Hemsl.	T	S	III
41	*Cimicifuga heracleifolia Kom.*	S	T	II
42	*Symplocarpus renifolius Schott ex Miq.*	S	T	II
43	*Primula sieboldii E.Morren*	T	S	III
44	*Veratrum maackii* var. *japonicum* (Baker) T.Schmizu	S	T	II
45	*Schisandra chinensis* (Turcz.) Baill.	T	S	III
46	*Syneilesis palmata* (Thunb.) Maxim.	T	T	I
47	*Thalictrum actaefolium* var. *brevistylum Nakai*	T	S	III
48	*Convallaria keiskei Miq.*	T	T	I
49	*Hosta capitata* (Koidz.) Nakai	T	S	III
50	*Ajuga spectabilis Nakai*	T	S	III
51	*Thalictrum uchiyamai Nakai*	T	S	III
52	*Potentilla stolonifera* var. *quelpaertensis Nakai*	S	T	II
53	*Asarum sieboldii Miq.*	T	S	III
54	*Hosta clausa Nakai*	T	S	III
55	*Lilium leichtlinii* var. *maximowiczii* (Regel) Baker	T	T	I
56	*Valeriana fauriei Briq.*	T	T	I
57	*Angelica gigas Nakai*	T	T	I
58	*Rhododendron schlippenbachii Maxim.*	T	T	I
59	*Matteuccia struthiopteris* (L.) Tod.	S	S	IV
60	*Polygonatum stenophyllum Maxim.*	S	S	IV
61	*Maianthemum dilatatum* (Wood) A.Nelson & J.F.Macbr.	T	T	I
62	*Sedum latiovalifolium Y.N.Lee*	S	S	IV
63	*Cryptotaenia japonica Hassk.*	T	T	I
64	*Hylomecon vernalis Maxim.*	T	T	I
65	*Pulsatilla koreana* (Yabe ex Nakai) Nakai ex Nakai	T	S	III
66	*Chloranthus japonicus Siebold*	T	T	I
67	*Euonymus alatus* (Thunb.) Siebold	T	S	III
68	*Hosta longipes* f. *alba* (Nakai) T.B.Lee	T	T	I

Rice varieties which are highly sensitive to submergence significantly decreased the ETo/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.
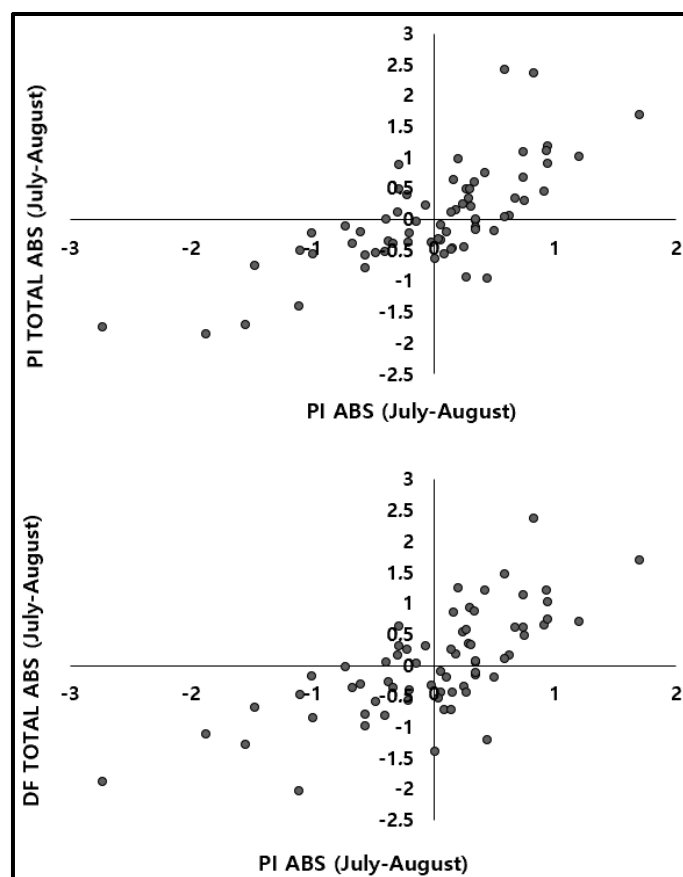
Fig. 2. Relationship of performance indice for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors

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