Sensing Plant Physiology and Environmental Stress by Automatically Tracking F_j and F_i Features in PSII Chlorophyll Fluorescence Induction

Qian Xia^{1,2}, Jinglu Tan³, Shengyang Cheng¹, Yongnian Jiang⁴, and Ya Guo^{1,2,3*}

1. Key Laboratory of Advanced Process Control for Light Industry, Ministry of Education, Jiangnan University, Wuxi 214122, China

2. School of Internet of Things, Jiangnan University, Wuxi 214122, China

3. Department of Bioengineering, University of Missouri, Columbia, MO 65211, USA

4. Jiangsu Zhongnong IoT Technology Co., LTD, Yixing 214200, China

* Corresponding: guoy@jiangnan.edu.cn; guoy@missouri.edu

Abstract

Following a step excitation, chlorophyll fluorescence (ChlF) from photosystem II of a dark-adapted plant leaf exhibits the well-known OJIP pattern. The OJIP induction has been widely applied in plant science, agriculture engineering, and environmental engineering. While the J and I phases are related to transitions of photochemical reaction redox states, characteristic fluorescence intensities for the two phases (F_j and F_i) are often treated as fixed time points in routine measurement and thus do not account for variations in plant and experimental conditions, which (1) neglects the time differences, potentially useful information for characterizing plant status and environmental factors, and (2) leads to errors in measured F_j and F_i values. In this work, a method for consistent measurement of F_j and F_i was developed through polynomial fitting and curvature analysis. The method measures the curvatures in the OJIP curve and automatically tracks the characteristic transition points under variable sample and experimental conditions. Experiments were carried out to demonstrate the concept and classification capabilities of the developed method. This research established a new framework to analyze ChlF and enhanced the applications of ChlF.

Keywords: Chlorophyll Fluorescence; Photosynthesis; Photosystem II; Polynomial Fitting; Curvature Analysis

1. Introduction

Light absorbed by plant photosystem II (PSII) in the photosynthetic process has three subsequent pathways: photochemical reactions, heat and chlorophyll fluorescence (ChlF) (Goltsev et al., 2003; Krause and Weis, 1991; Lavergne and Trissl, 1995; Stirbet et al., 1998; Vredenberg, 2004; Taiz and Zeiger, 2006). Since the emission of ChlF competes for light energy with the other two pathways (Lubitz et al., 2008), almost all the changes in photosynthesis can be reflected by PSII ChlF (Zhu et al., 2005; Rodriguez & Greenbaum, 2009). ChlF measurement is thus a reliable method to study plant physiology and environment factors that influence photosynthesis (Mohammed et al., 1995; Maxwell & Johnson, 2000; Coombs & Long, 2014). In addition, ChlF is a fast, noninvasive, simple, and intuitive way to represent the changes in photosynthetic activities (Kooten et al., 1990; Mathur et al., 2011).

When a step illumination is applied, the intensity of PSII ChlF from a dark-adapted plant leaf changes with time in a unique pattern of the commonly-labeled O-J-I-P phases, which is referred to as the ChlF induction curve or OJIP curve. The OJIP curve contains abundant information about the process of photosynthesis: the absorption and conversion of light energy, the transfer and distribution of energy, the state of the reaction centers, the activities of the PSII donors and the receptors, plastoquinone (PQ) pool size and the activities, excess light energy and its dissipation, photosynthetic light suppression, and light damage. ChlF induction has been extensively used in the literature to analyze plant photosynthesis and physiological conditions (Ogaya et al., 2011; Schansker et al., 2014; Guo & Tan, 2015; Guo et al., 2015).

There are four important points on an OJIP curve. O reflects the initial fluorescence when a leaf is exposed to light after dark adaptation. J indicates accumulation of plastoquinone A (Q_A^-) (Strasser et al., 1995). I is related to the heterogeneity of the PQ pool (Strasser et al., 1995; Jee, 1995). P shows the maximum value of fluorescence. F_j and F_i represent the ChlF intensity during the J and I phases of the ChlF kinetics, respectively. Obviously, these time points depend on the photochemical reaction kinetics, which implies that differences in plant physiological and experimental conditions may lead to different times of occurrence for these phases. J and I are generally defined as the first and the second inflection points or intermediary peaks on the ChlF induction curve, respectively (see Figure 1). Different plant species, light intensity, temperature, salinity, and drought may affect the plant physiological status (D'Ambrosio et al., 2006; Koyro, 2006; Ruban & Belgio, 2014; Guo & Tan, 2015) and thus the times of occurrence of these transitions.

The direct ChlF induction parameters include F_o , F_j , F_i , and F_m . Many other ChlF parameters can be computed from these four. F_o is defined as the initial fluorescence and thus it can be measured at a fixed time after the initiation of excitation. F_m represents the maximum fluorescence in the entire ChlF induction curve and thus the exact time of F_m measurement is not critical. J and I, however, are transient phases between O and P. They may occur at different times depending on the plant species, illumination, and growth environments. Currently, all commercial ChlF instruments measured F_j and F_i at a fixed predefined time, although it is manually adjustable. The instruments cannot automatically adjust the characteristic time and track the F_j and F_i points. Although users may configure the predefined time through operating the instrument menu, but it is boring and time consuming to set it for each measurement and ChlF features were usually read according to a fixed time. This has several obvious drawbacks: (1) It neglects the time differences, which may be useful information for characterizing plant status and environmental factors because these times of occurrence reflect the reaction rates, and (2) It leads to errors in measured F_j and F_i values because a fixed time is inappropriate for all plant and experimental conditions. This may limit the ChlF usefulness and results in discrepancies in interpretation of ChlF kinetics.

This work was aimed at developing a method to determine the J and I characteristic times adaptively and consistently when plant and experimental conditions vary. Based on the common interpretations of the ChlF induction kinetics, these times were determined according to the curvature changes on the OJIP curve. Least-squares polynomial fitting was used to filter experimental data. Comparisons were made between the proposed method and the traditional method. Applications were used to validate the usefulness of the proposed method.

2. Method Development

A typical OJIP induction curve is shown in Figure 1. Since J and I are inflection points in ChlF intensity resulting from changes in forward (downstream) reactions (Strasser et al., 1995; Stirbet and Govindjee, 1992), they are simply points of curvature changes in the induction curve. Consequently, they can be conveniently and consistently located by finding the local curvature maxima on the induction curve. Because the induction curve is commonly observed in semi-log scale, curvature is computed based on logarithm of time.

Fig. 1. A typical ChlF induction or OJIP curve

The curvature of a curve can be easily found by computing numerical differences, but numerical differences of a measured curve easily suffer from noise, resulting in difficulty in finding the true local maxima of curvature. To eliminate the influence of noise, the induction curve may be first fitted with a spline or polynomial of the form.

$$y = \sum_{n=0}^N a_n x^n \quad (1)$$

where y is ChlF, x is the logarithm of time (as the induction curve is usually presented in semi-log scale to reveal J and I), N is the order of the fitted polynomial, a_n ($n = 0 \dots N$) are coefficients, and n is an integer.

From Eq. (1), the first derivative of y with respect to x can be computed as:

$$\dot{y} = \sum_{n=1}^N a_n n x^{n-1} \quad (2)$$

and the second order derivative $\ddot{y}$ is:

$$\ddot{y} = \sum_{n=2}^N a_n n(n-1) x^{n-2} \quad (3)$$

Curvature k is computed as:

$$k = \frac{|\ddot{y}|}{\sqrt{(1 + \dot{y}^2)^3}} \quad (4)$$

In this work, N was experimentally determined. When N increases, the fitting error for Eq. (1) will decrease. The N -value where the fitting error starts to level off was selected as the desired value and it was 15 in this work. The polynomial fitting is used to capture the main variations in ChlF and thus to eliminate noise. As a result, the exact order of the polynomial will not affect the analysis and the computed derivatives significantly. When the curvature k is obtained, it is easy to determine the times of J and I based on the y (OJIP) pattern and the k pattern. Obviously, either y or k may be rescaled or shifted for easy identification of the maxima without affecting the final outcomes.

3. Experiments

3.1 Plant Samples

Three types of tree leaves (*Cercis chinensis*, *Pittosporum tobira* and *Elaeocarpus glabripetalus merr*) and two types of vegetable leaves (spinach and lettuce) were used in the experiments.

The tree leaves were collected from the campus of Jiangnan University (Wuxi, China). The leaves were picked in the morning between 6 am and 7 am in July, when the environment temperature was around 28°C. The picked tree leaves were from the middle of the tree canopy on the south side. The two types of vegetables were acquired from a local farmers' market in an early morning in July with an environmental temperature at approximately 28°C. The spinach and lettuce leaves are intact and fresh. The spinach had roots but the lettuce roots had been cut. The samples were transported to the laboratory for experiments. The laboratory temperature was at 25°C.

In order to reduce evaporation and influence of water status on ChlF emission, all the sample leaves were floated in water for at least half a day. Before ChlF measurement, the leaves were dark-adapted for at least 30 minutes in dark-adaption clips. ChlF was measured with a FluorPen PSI (Photon Systems Instruments, Czech Republic) by selecting its OJIP protocol.

3.2 Experimental Design and Data Analysis

In this work, four sets of experiments were designed to demonstrate the concept and usefulness of the proposed method. Twenty-nine leaves were measured for each variety of plant in the first three sets of experiments. For the fourth set of experiments, fourteen leaves of each plant type were measured.

The first set of experiments involved all five different types of leaves and was used to show the differences in the times of occurrence and F_j and F_i values from different plants obtained by the developed method or the default ChlF meter protocol. The illumination light intensity was set as 3,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$.

The second set of experiments included four different leaves (spinach, *Cercis chinensis*, *Pittosporum tobira* and *Elaeocarpus glabripetalus merr*) and was used to compare the differences in the times of occurrence and F_j and F_i values under varied light intensities obtained by the developed method or by the default ChlF meter protocol. Three levels of light intensity were used: 750 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, 1,500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, and 3,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Half an hour dark-adaptation was applied in between measurements.

The third set of experiments involved the three types of tree leaves and was used to compare the differences in the times of occurrence and F_j and F_i values in various temperatures obtained by the developed method or by the default ChlF meter protocol. Each leaf was cut into two halves along the central vein. The leaf halves were considered to be in similar physiological status. One half was placed in water at 23°C and the other in water at 33°C. They were all processed at the same time. The illumination light intensity was set at 3,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$.

The fourth set of experiments involved all five plant types and was used to compare the differences in the time of occurrence and F_j and F_i value under different levels of detachment stress obtained by the developed method or by the default ChlF meter protocol. All the leaves in this set of experiments were kept in 2°C. Wet paper towels were applied on both sides of each leave sample to keep it moist (Guo et al., 2015). ChlF was measured each morning and night in 12-hour intervals five times. The illumination light intensity was set at 3,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$.

Polynomial fitting and curvature analysis were executed in Matlab (Mathworks, Natick, MA, USA). Statistical analysis was also performed to detect statistical significances in SPSS (Armonk, NY, USA).

4. Results

4.1 Curve Fitting and Curvature Analysis

Examples of polynomial fitting to experimental data are shown in Fig. 2. Fig. 2a is for a tree (*Pittosporum tobira*) leaf and Fig. 2b is for a spinach leaf. For a total of 722 sets of OJIP data, the relative fitting error is less than 0.018%, which shows that polynomials can represent the OJIP patterns very well.

Fig. 2. Comparison between experimental data and fitted polynomial. (a) A tree leaf (*Pittosporum tobira*), (b) A spinach leaf.

Figure 3 shows OJIP induction curves and the corresponding curvature values obtained with Eq. (4). Fig. 3a is the result for a tree (*Pittosporum tobira*) leaf and Fig. 3b for a spinach leaf. Obviously, there is a maximum value for each transition on the OJIP induction curve. These maximums provide quantitative information on OJIP characteristic transitions. The maxima around J and I appear in pairs: one corresponds

to the upward transition and the other to the downward transition. Based on the descriptions of J and I in the literature, the maxima corresponding to the upward transitions are J or I. The peaks in the curvature are useful for quantitatively segmenting the OJIP curve into different phases.

Fig. 3. OJIP induction curve and corresponding curvature. (a) Result for a tree leaf (*Pittosporum tobira*); (b) Result for a spinach leaf.

As explained earlier, different samples may differ in physiological states, which may result in earlier or later times of occurrence for J and I. Even for the same leaf, if the environmental conditions such as temperature and illumination light intensity are changed, the J and I transitions may appear earlier or later. For a given leaf, it is difficult to determine what the optimal illumination light is before experiments. During experiments, light illumination changes the dark-adaptation status and thus it is difficult to try different experimental conditions. This causes errors in determining J and I transitions if a fixed time is used in ChlF meter protocols. Figures 4a and 4b show comparisons of the J and I transitions determined at the default times or by the curvature analysis. Figure 4a is for the leaf of *Cercis chinensis* at various temperatures and Fig. 4b is the result for *Cercis chinensis* at various light intensities. It is clear from Figures 4a and 4b that the curvature analysis method could track the transitions adaptively to always locate J and I at the inflection points as desired when the illumination light intensity and environmental temperatures change, whereas the traditional method of a fixed time misses the inflection points most times and by variable amounts. For all the measured *Cercis chinensis* samples, the time of occurrence for J (t_j) varied from 1,293.41 μs to 2,127.90 μs and that for I (t_i) varied from 30,734.79 μs to 47,941.69 μs . The default values in the instrumental protocol for J and I, however, are fixed at 2,012 μs and 30,321 μs , respectively. Distributions of the t_j and t_i determined by the developed method are shown in Figures 4c and 4d, respectively, which show considerable ranges of variation and thus inadequacy of using fixed times.

Fig. 4. Comparisons of the fixed J and I transition points in instrumental protocol and those determined by the curvature analysis method. (a) Results for different light intensities (750, 1,500, and 3,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$), (b) Results for different temperatures, (c) Distribution of t_j for different light intensities, (d) Distribution of t_i for different light intensities, (e) Distribution of t_j for different temperatures, (f) Distribution of t_i for different temperatures.

4.2 Differences in the times of occurrence and F_j and F_i values from different plants obtained by the developed method or the default ChlF meter protocol

The p -values (Appendix A) were examined for comparing the J and I times of occurrence and ChlF intensities obtained through the traditional method (F_j , T_j , F_i , T_i) and those by the developed method (f_j , t_j , f_i , t_i). The results reveal that the F_j and f_j , T_j and t_j are statistically different for all the five types of plant leaves and that F_i and f_i ,

255 T_i and t_i are statistically different for some of the five types of plant leaves.

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257 To show the effects of plant type, the ChlF variables obtained by the traditional
258 method and those by the developed method were subject to t-tests (Appendix B). It
259 should be mentioned that T_j and T_i were fixed and thus were not subject to the
260 analyses. The results shown in Appendix B indicate that the measurements by the two
261 methods differed significantly for the plant species in most cases. It is important to
262 note that t_j is significantly different for all the five species and yet it is taken as a
263 single fixed value in the traditional method.

264

265 From this set of experiments, it is clear that the proposed approach gave more
266 consistent differences among different plant types. t_j , in particular, consistently
267 differed among all five plant types, whereas the traditional method uses a fixed value.

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269 *4.3 Differences in the times of occurrence and F_j and F_i values under varied light*
270 *intensities obtained by the developed method or by the default ChlF meter protocol*

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272 Appendix C shows the means of ChlF variables determined by the default ChlF meter
273 protocol or the developed method for the four types of leaves under different light
274 intensities. Appendix D shows the p values of statistical comparison between the two
275 methods under different light intensities, which reveal that most of the ChlF variables
276 are statistically different. With increasing light intensity, the J and I points apparently
277 moved to the left in general as revealed in Figure 4a and the t_j values shown in
278 Appendix C. The times of occurrence for J and I varied significantly among different
279 plant types and light intensities. However, in traditional instrumental protocol, t_j and t_i
280 usually were set at fixed values. This unavoidably leads to discrepancies.

281

282 *4.4 Differences in the times of occurrence and F_j and F_i values in various*
283 *temperatures obtained by the developed method or by the default ChlF meter protocol*

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285 Appendix E shows the mean values of ChlF variables determined by the developed
286 method or by the traditional method in room temperature (23°C) or an elevated
287 temperature (33°C) and Appendix F shows the statistical comparison. Clearly,
288 temperature has a significant influence on the occurring times of J and I transitions as
289 revealed in Appendix D and Figure 4b. The developed method could automatically
290 track the J and I transitions according to the curvature of the OJIP induction.
291 Appendix E also reveals that lower temperature may result in higher values for t_i and
292 t_j . In this set of experiments, the J and I points apparently moved to the left and the
293 obtained t_j and t_i values increased with the increments of temperature.

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295 *4.5 Differences in the times of occurrence and F_j and F_i values under different*
296 *detachment and chilling stress obtained by the developed method or by the default*
297 *ChlF meter protocol*

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To compare the differentiation abilities between the developed method and the default ChlF meter protocol, measurements from spinach leaves in the fourth set of experiments were used for statistical analysis and the results are shown in Appendix G. The leaves measured were subjected to five levels of detachment duration and chilling stress. In this group of experiments, the J and I points apparently moved to the right with the increase of duration of detachment and chilling stress. It can be easily found that all the statistically significant differences detected by the traditional method were detected by the corresponding developed method. However, the developed method especially t_j and t_i , differentiated more group differences than the traditional method. t_j and t_i are unjustifiably set as constants in the traditional method.

5. Discussion

Differences in plant physiological status and experimental conditions such as illumination intensity and temperature will lead to significant changes in the shape of the OJIP induction curve and shift the J and I points either left or right. This was well demonstrated by the experiments in this work. A standard instrumental protocol uses fixed occurring times for the J and I transitions, which may catch the right J and I transition times for a specific plant type and under standardized conditions but may miss the inflection points by large margins under other circumstances as shown in Figure 4. This will lead to inconsistencies in measurement and introduce additional variations, which could reduce the usefulness of ChlF measurements.

The developed method based on curvature analysis can automatically locate J and I when differences in plant physiological status and environmental conditions shift the inflection points. ChlF measurements by the proposed method show better consistency and differentiation capabilities than those by the default instrument protocol. For example, f_j obtained by developed method showed consistent differences between the 2nd morning and the 3rd night for chilled spinach while the traditional method could not detect the difference.

Statistical comparison between the two methods under different light intensities, different temperatures and different plants show that there are statistically significant differences in most of the ChlF variables. The experimental results by the new method are consistent with what is known. For example, photosynthesis will speed up when the light intensity increases and faster reaction rates will lead to earlier J and I transitions, as shown by the new method.

The experimental results also show that t_j and t_i are very useful ChlF variables. t_j can clearly differentiate five types of leaves but in the standard method it is set as a fixed value. t_i and t_j can also be used to distinguish plants in different light intensities and different temperatures. While one may argue that the curvature method changes what have been conventionally understood as the J or I points, these new characterizations could be labeled differently and used because their differentiation capabilities as demonstrated in this work. Furthermore, this work indicates that other mathematically-definable features of the ChlF signal could prove useful as well.

6. Conclusion

PSII chlorophyll fluorescence characteristics have been widely used to sense plant physiological status and environmental changes. Differences in plant and experimental conditions will unavoidably result in different transition times. The method developed in this work can automatically detect and track the transitions. The measured characteristic fluorescence levels showed better differentiation abilities than those determined by the standard ChlF meter protocol method. The transition times determined show strong differentiation abilities for different plant species under different experimental conditions.

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Fig. 2. Comparison between experimental data and fitted polynomial. (a) A tree leaf (*Pittosporum tobira*), (b) A spinach leaf.

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Distribution of t_i for different temperatures.