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A novel method for measuring photosynthesis using delayed fluorescence of chloroplast

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Abstract

Photosynthesis is the most important chemical reaction in the world. The measurement of plant photosynthesis rate plays an important role in agriculture. Light-induced delayed fluorescence (DF) in plants is an intrinsic label of the efficiency of charge separation at P_{680} in photosystem II (PS II). In this paper, we have developed a biosensor that can accurately measure the plant photosynthesis ability by means of DF. Compared with common methods for measuring the photosynthesis rate based on consumption of CO_2, the proposed technique can quantify the plant photosynthesis ability with less influence of the environment. The biosensor is an all-weather measuring instrument, it has its own illumination power and utilizes intrinsic DF as the measurement marker. The current investigation has revealed that, there is a good correspondence between the results measured by the biosensor and that by commercially available portable photosynthesis system under controlled conditions. We thus conclude that DF is an excellent marker for evaluating plant photosynthesis ability under its biological status with less interferences of the environment.

Keywords: Biosensor; Delayed fluorescence; Photosynthesis ability; Photosynthesis rate

1. Introduction

Solar energy is the most important energy source for life-actions on earth. Photosynthesis is the only way by which solar energy can be stored by plants. Photosynthesis, as the most important chemical reaction (Shen, 2000b), can affect most aspects of life forms on earth. Extensive investigations have been conducted for improving the photosynthesis rate by controlling environmental factors. There are three types of methods for quantifying photosynthesis rate by measuring the rates of CO_2 consumption, O_2 evolution and increment for leaves' dry matter (Shen, 2000a).

Most commercially available instruments for measuring photosynthesis rate, such as the prevalent LI-6XXX series of portable photosynthesis system (LI-COR, USA), are based on CO_2 consumption. The measurement is affected by environmental factors, such as light intensity, temperature, humidity and CO_2 concentration, etc. Variations in these factors would cause substantial differences in the measurement results. The chloroplast is an organelle of plant cell. All photosynthesis processes (light absorption, charge separation, electron transport, photophosphorylation, CO_2 fixation, and C-4 Pathway, etc.) are carried out in the chloroplast.

Photosynthesis II (PS II) is regarded as the location for primary photochemical reactions, where the charge separation, water photooxidation to evolve oxygen and to produce electrons and protons occur. The physiological states of chloroplasts would determine the ability of plant photosynthesis rate (Yu and Tang, 1998).

The delayed fluorescence (DF) phenomena can be described as light emission of photosynthetic organisms shortly after their illumination. DF shares the same spectrum of ordinary fluorescence, but occurs with a time delay (from milliseconds to minute) (Strehler and Arnold, 1951). However, both fluorescence and DF are radiated during the same excited P^*_{680} (P^*_{680}) to P_{680} transition. The difference is that, for fluorescence, P^*_{680} is created directly by light excitation, while for DF, the P^*_{680} state is a results of recombination of products formed in a primary photochemical reaction. Because a light-induce DF happens later than fluorescence

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and its origin is associated with photosynthesis, it is more closely related to photosynthesis than chlorophyll fluorescence (Strehler and Arnold, 1951; Itoh and Murata, 1973; Chaerle et al., 2001). Loss of chlorophyll is the external manifestation of the onset for leaf senescence. Decrease in the thylakoid protein content (Misra and Biswal, 1980) and the photochemical efficiency of photosystem II (Misra and Biswal, 1982). Inactivation of the oxygen evolution system (Margulies et al., 1971), release of manganese (Mn) and degradation of D1 polypeptide (Roberts et al., 1984) have been reported. The chlorophyll content is directly related to the plant physiological states and functions. Because there exists a linear relationship between the DF intensity and chlorophyll content within a limited range (Gunasekaran, 1990), the energy conversion in photosynthesis can be evaluated by quantifying DF (Anderson and Boardman, 1996; Gunasekaran, 1990; Fritz-Albert, 2001). The changes in the rate of transport through the phosphate translocator are clearly manifested in induction kinetics of DF (Edwards and Walker, 1983). Research on the complex behaviors of the DF (Gerhardt and Bodemer, 2000) has led to the conclusion that DF can be used to determine concentrations of photosynthetically active pigments and to analyses phytoplankton compositions in freshwaters (Bodemer, 1998; Bodemer et al., 2000). Accordingly, monitoring DF provides a suitable tool to analyze the back reactions of trapped redox equivalents in PS II. DF is not only an intrinsic fluorescence label of the efficiency of charge separation at P_{680}, but also a tool to differentiate if a photosynthesis process happens in antenna or in electron transport chain. Therefore, the researches on DF provide one important method for express analysis of the plant photosynthesis ability.

In this paper, according to maximum intensity of DF, excitation parameters of DF have been optimized experimentally. Based on the excitation parameters, a biosensor for photosynthetic ability is proposed. Compared with common methods for measuring the photosynthesis rate based on consumption of CO_2, the proposed biosensor can quantify the plant photosynthesis ability with less influence of the environment. The current investigation has revealed that, there is a good correspondence between the results measured by the biosensor and that by commercially available portable photosynthesis system under controlled conditions.

2. Experiment

2.1. The preparation of chloroplasts

The chloroplasts were prepared according to Ye et al. (1995). Clean spinach (*Spinacia oleracea*) leaves without main vein were crushed with buffer (0.2 M NaCl, 0.1 M Sucrose, 0.05 M Phosphate, pH 7.4). The crushed mixture was filtered with 4 and 16-fold gauzes. The filtered liquid was centrifuged for 5 min at 600 G after removing the precipitate. The supernatant was further centrifuged for 10 min at 500 G. The precipitate (chloroplasts) was dissolved in isotonic solutions (0.05 M NaCl, 0.3 M Sucrose, 0.05 M Phosphate, pH 6.9). Entire process was at 4 °C, in a dark environment. The isolated chloroplasts were stored in liquid N_2.

2.2. Spectrum measurement

Both emission spectrum and excitation spectrum of DF were obtained by using a LS-55 Luminescence Spectrometer (Perkin-Elmer, USA) with photomultiplier (PMT) (Model R-928, Hamamatsu Photonics K.K., Japan). The PMT is sensitive within spectral range between 400 and 900 nm. The data were transferred to a personal computer (Intel, USA). The experiment was carried out at room temperature.

2.3. Delayed fluorescence sensing system

The DF detection system was custom-built in our laboratory, based on an intensified CCD (ICCD-576-S/1, −40 °C, Princeton Instruments, USA). The diagram of the system is shown in Fig. 1. A He-Ne laser (632.8 nm) was used as the excitation-light source. The output of the laser was coupled into an optical fiber with a distal microlens for homogeneous superficial irradiation of the leaves. The irradiation fluence rate was adjusted by changing the irradiation field size while keeping the output power of the laser constant. The entire setup was housed in a light-tight chamber. Each sample was stabilized inside the chamber for 10 min before a measurement started. DF from the sample, after passing a 685 nm band filter, was collected by a Nikon photographic lens (50 mm, F/1.8) and projected onto a micro channel plate (MCP) and cooled CCD assembly. The output signal was collected by a PC and LabVIEW via a ST-130 (Princeton Instruments) controller. The samples were irradiated by laser light for various length of time. The data collection started at 0.5 s upon the completion of the light irradiation, and lasted for 60 s. Images were collected every 3 s. The digitized image was processed by WinView software for the intensities of whole leaf regions from each image. Total DF, defined as the cumulated photon counts between 0.25×10^{-3} and 60 s after irradiation, the data was fit for a double exponent decay function using software ORIGIN. The function was

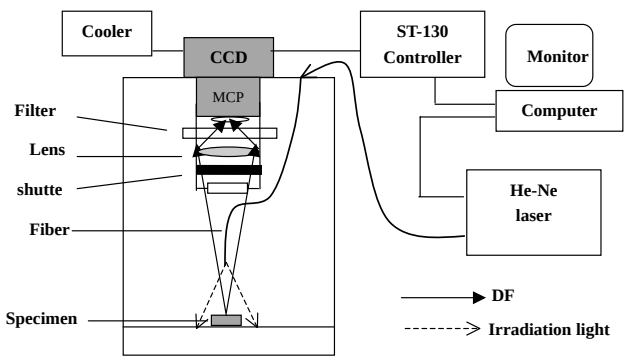


Fig. 1. Schematic diagram of experimental setup.

integrated between 0.25×10^{-3} and 60 s for the DF of the sample. The experiment was carried out at 28 °C.

2.4. Validation of the plant photosynthesis rate measurement

To validate our plant photosynthesis ability measurement system, the results were compared to that from a commercially available system (Model: LI-6200 LI-COR, USA, courtesy of Photosynthesis Center of Life Science Institute, South China Normal University). The comparisons were made both in the field and in a custom-built climate chamber. The field study was conducted in a commercial spinach field near Guangzhou, China. Samples (10 pieces of secondary full-grown spinach leaves) were randomly selected from the field and measured with both the DF system and the commercial unit. The same samples were then harvested and bought back to the laboratory and measured in a climate chamber. To control the environment, the climate chamber was equipped with a dew point producer (Model: LI-610, LI-COR, USA) for relative humidity, and an adjustable tungsten lamp (maximum intensity: 5000 lx) for artificial illumination. The chamber was operated at a stable environmental temperature (28 °C). The CO_2 concentration within the chamber was monitored with the custom-built CO_2 concentration gauge of the commercial unit and was stable between 320 and 330 ppm throughout the study.

3. Results and discussion

3.1. The choices of optimal excitation parameter and measurement temperature

3.1.1. Emission spectrum and excitation spectrum of DF

Fig. 2A shows a DF emission spectrum. We can see that the emission spectrum consists of a main peak at 685 nm. Fig. 2B shows a DF excitation spectrum (685 nm) of spinach chloroplast at room temperature. The profile of the excitation spectrum of DF matches the absorption spectrum of the antenna pigments (unpublished data). Within visible light wavelength, the excitation spectrum of DF is nearly identical to that of the action spectrum of photosynthesis (Pan and Dong, 2002). With spinach chloroplast as our sample, we have found that lamina and its chloroplast had identical excitation DF spectra.

Fig. 2B also shows that, within the visible light spectrum, the excitation efficiency of DF is similar for the blue and red region, but significantly lower within the green region. This result is comparable to what reported by Emerson about the photosynthesis efficiency caused by light of different wavelengths. Therefore, the DF emission and photosynthesis are likely to share the same energy resources; without continuous illumination, the energy that has been absorbed by a plant will not be progressed into photosynthesis, but released in the form of DF.

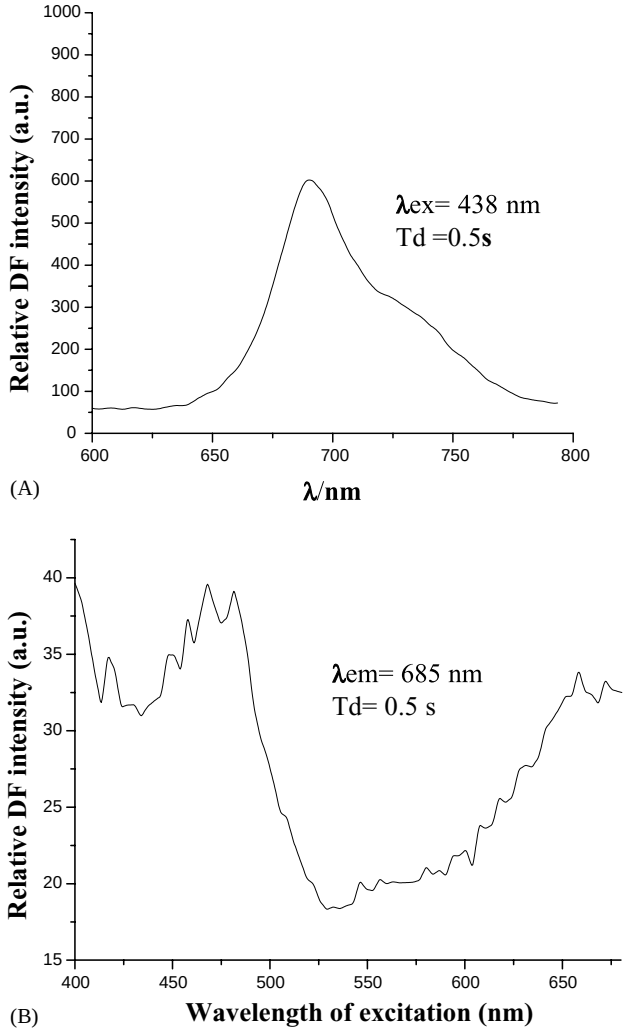
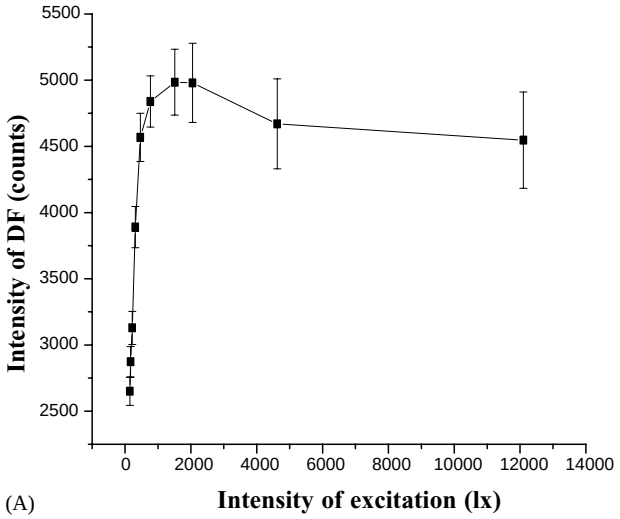


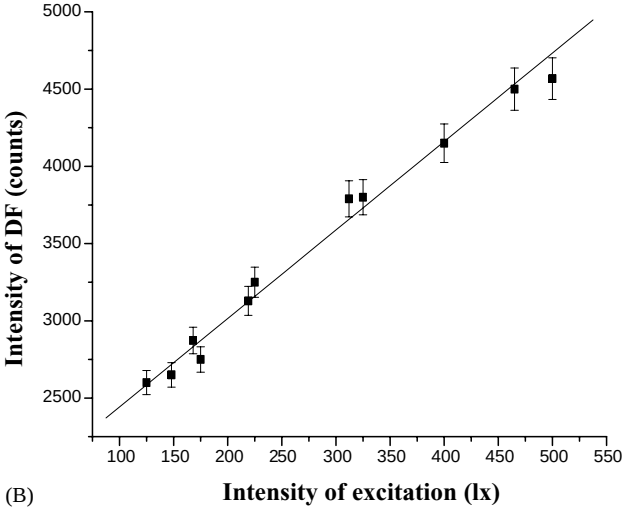
Fig. 2. DF emission spectrum (A) and excitation spectrum (B) of spinach chloroplasts (response was measured at 0.5 s after excitation, slit: ex = 5 nm, em = 10 nm, chloroplast concentration: 10 μm/l).

3.1.2. The relationship between the excitation intensity and the DF intensity

Fig. 3 shows the relationship between intensities of the He-Ne laser excitation light and the resulted DF. The intensities are linearly correlated when the irradiation intensity is low, as shown in Fig. 3B (an expanded section of Fig. 3A). As the irradiation intensity increases above certain limit, DF signal gradually reaches saturation. Further increase in the irradiation intensity may even have negative effect on DF intensity, although this is not statistically confirmed (Fig. 3A). By strategically setting the irradiation light intensity at the saturation point will result in both maximum and stable DF signal. It is noticed that the total excitation energy required to obtain maximum DF is different for each plant. For spinach, the DF saturation is reached at 1800 lx at 632.8 nm.



(A)



(B)

Fig. 3. Effect of excitation intensity on DF (excitation illumination time: 4 s).

3.1.3. The relationship between the excitation times and the DF intensity

By setting the He-Ne laser intensity at 2000 lx, the DF intensity as a function of light irradiation time has been studied. The results are shown in Fig. 4. Maximum DF intensity is reached within the first 3–5 s. Prolonged excitation gradually degrades the DF intensity. Therefore, it is advisable to use short (about 3–5 s) but strong (to assure saturation) excitation for most efficient DF measurement.

3.1.4. The relationship between temperature and the DF intensity

Fig. 5 shows the relationship between sample temperature and the intensity of DF. Maximum DF intensity is observed at 28 °C. This peak temperature varies for different plants. The dependence of DF intensity on temperature is nearly identical to that of the photosynthesis (Shen, 2000b). The finding confirms that, the temperature dependence of DF is not a major problem in case of agricultural products encountering relatively small temperature changes (Jacob et al., 1965).

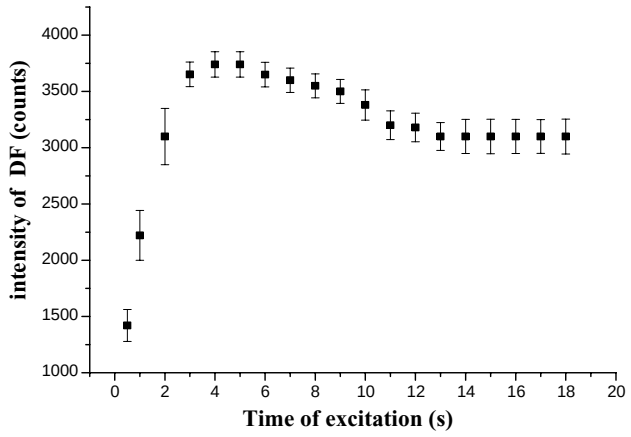


Fig. 4. DF intensity as a function of excitation time (illumination intensity: 2000 lx).

3.2. Correlation analyses between the biosensor results and that from traditional methods

3.2.1. Field study

The sensor of the DF system was set at the optimal excitation parameters obtained from the experiments (excitation wavelength: 632.8 nm, excitation intensity: 2000 lx; excitation time: 4 s, temperature 28 °C). Fig. 6A shows the DF intensity measured using the DF system, and the corresponding Pn (net photosynthesis rate) measured by the commercial unit. Statistical analysis shows a significant correlation between the DF intensity and Pn in the field study ($R^2 = 0.959$, Fig. 6B).

3.2.2. Bench study

The same leaf samples were studied in the controlled climate chamber (illumination intensity: 2000 lx, relative humidity: 80%, CO_2 concentration: 320–330 ppm, temperature

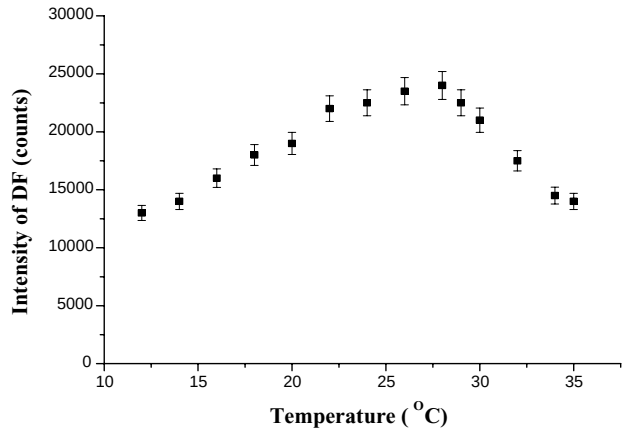
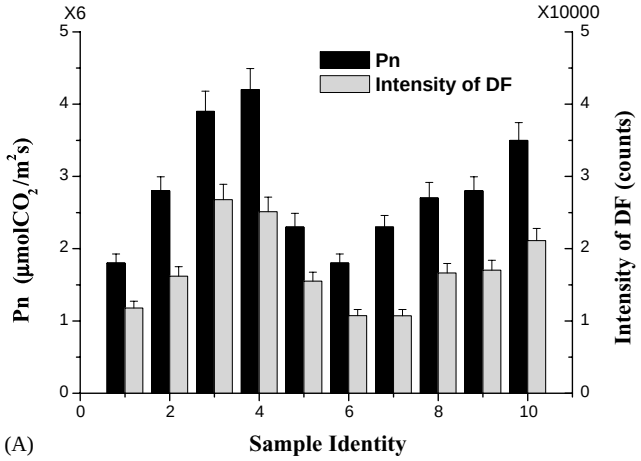
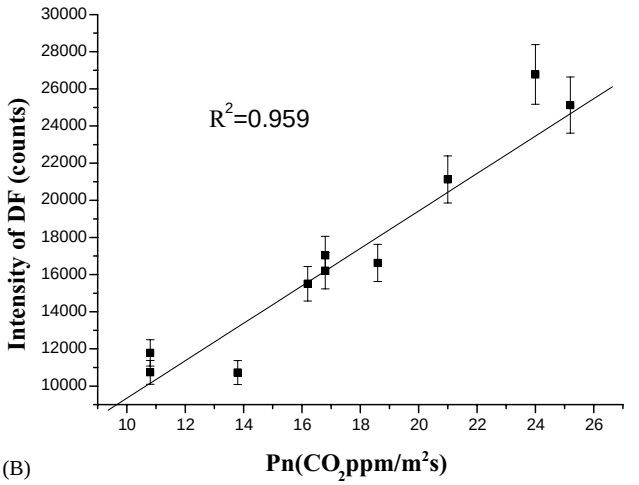


Fig. 5. Effect of temperature on DF (excitation illumination time: 4 s, illumination intensity: 2000 lx).

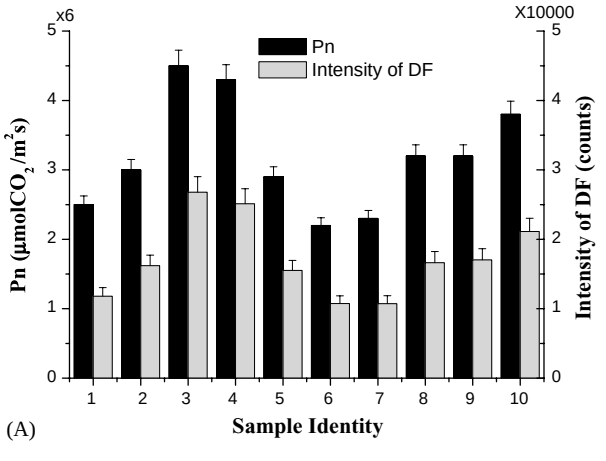


(A)

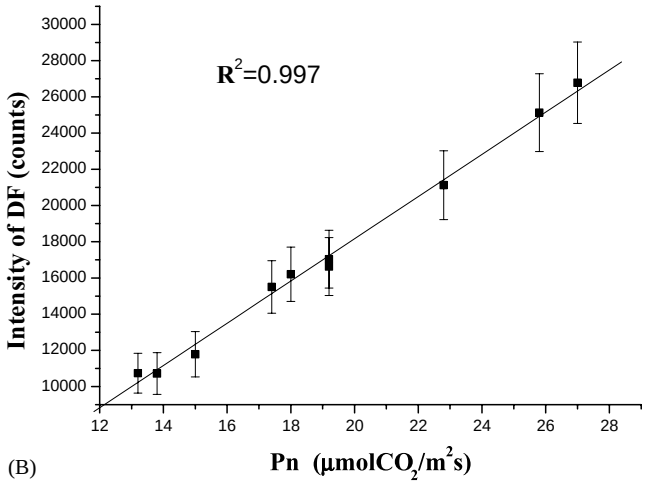


(B)

Fig. 6. (A) comparison between Pn and DF intensity measured in the field and (B) correlation between field Pn and DF intensities ($R^2 = 0.959$, $P < 0.001$).



(A)



(B)

Fig. 7. (A) comparison between Pn and DF intensities under laboratory conditions (illumination intensity: 2000 lx, CO_2: 320–330 ppm, humidity: 80%, temperature: 28 °C) and (B) correlation between Pn and DF intensity under laboratory conditions ($R^2 = 0.997$, $P < 0.0001$).

28 °C). Both DF system and the commercial unit were set up in the identical ways as in the filed study. The results are shown in Fig. 7A. Statistical analysis shows that, there is an excellent correlation between the DF intensity and Pn in the bench study ($R^2 = 0.997$, Fig. 7B).

By comparing Figs. 6B and 7B, it is clear that, the linear correction between the DF intensity and Pn in a controlled climate is superior, compared to that in the field. The results also show that, for the same samples, the DF intensities are nearly identical as measured in the field and in the chamber. In contrary, the Pn values measured with the commercial unit in the field are generally lower than those in the chamber, and the differences between the two are overall random. This is likely due to the variations in environmental factors in the field, such as illumination intensity due to leaf positions, air humidity lowness, etc. The results indicate that, with its illumination power and utilize intrinsic DF as its measurement marker, the biosensor can accurately measure the plant photosynthesis ability with minimal influence of the environment.

With traditional method, photosynthesis ability is evaluated by measuring CO_2 consumption in unit time and area. With our biosensor, the photosynthesis ability is measured as the DF intensity with a pre-defined light excitation. DF is an intrinsic fluorescence label for the efficiency of charge separation at RC in PS II. Therefore, the measured results can express the total amount of separated charge generated by a unit area of a leaf when excited by the light. By using an excitation light intensity that saturates the DF, the signal fluctuation caused by varying environmental illumination condition is eliminated. The measurement results, thus, truly reflect the plant photosynthesis ability under its physiological condition.

4. Conclusion

In this paper, we have developed a biosensor that can accurately measure plant photosynthesis ability by means of DF. For the samples investigated, the results obtained by

our DF instrument were more accurate than the results obtained by an instrument based on CO_2 consumption. This instrument can more accurately reveal the plant photosynthesis ability with minimal environmental influence by using a customer-built illumination source and utilization of intrinsic DF. Therefore it is likely that the biosensor will likely to provide a new approach for measuring the plant photosynthesis ability.

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