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A new principle photosynthesis capacity biosensor based on quantitative measurement of delayed fluorescence *in vivo*

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Abstract

Delayed fluorescence (DF) is an excellent marker for evaluating plant photosynthesis. Compared with common methods for measuring the photosynthesis rate based on consumption of CO_2, DF technique can quantify the plant photosynthesis capacity more accurately and faster under its physiological status with less interference from the environment. We previously reported a method for measuring photosynthesis using DF of chloroplast [Wang, C.L., Xing, D., Chen, Q., 2004. Biosens. Bioelectron. 20, 454–459]. In the study, a novel fast and portable photosynthesis capacity biosensor system was developed, which was composed of light-emitting diode lattice as excitation light source, Channel Photomultiplier DC-Module to achieve DF, single-chip microcomputer as control center, hermetic dark sample chamber, battery power supply and CO_2, humidity and temperature controller. Compared with our previous work, the system was portable and can directly measure plant photosynthesis capacity *in vivo* in less than 10 s. A database in the software to carry out data acquisition and processing was developed to translate maximal DF intensity to net photosynthesis rate (Pn). In addition, local-control and remote-control mode can be chosen in the system. To demonstrate the utility of the system, it was applied to evaluate maximum Pn of four different plant species samples (Queen Rape Myrtle (var. rubra), soybean (Lu Hei No. 1), maize (Jin Dan No. 39) and rice (Jing Dao No. 21)) in field. The results were compared with that using commercial photosynthesis system LI-6400 and the uncertainty was less than ±5%. The new principle of photosynthesis measurement is a challenge and breakthrough to conventional method of gas exchange and may be a potential technique of next generation photosynthesis measurement.

Keywords: Photosynthesis capacity; Delayed fluorescence; Biosensor; *In vivo*

1. Introduction

Based on the theory of higher plant delayed fluorescence (DF) developed during last some years, it is possible to determine such characteristics of primary processes as rate constants for forward electron transport reactions and activation energies for backward electron transport reactions in the photo system (PS) reaction center (RC), the charge location in the RC and the state of Calvin cycle (Sane and Rutherford, 1986; Khuznetsova et al., 2001). Earlier, it has been shown that DF is a sensitive test for the state of photosynthetic system. Even the changes in the rate of transport through the phosphate translocator are clearly manifested in induction kinetics of DF (Rutherford and Inoue, 1984; Badretdinov et al., 2004). The sensitivity of DF to changes in metabolism coupled with the ease and rapidity that measurements of DF can be made makes DF potentially useful for noninvasive diagnosing the photosynthetic performance of plants *in vivo*.

DF of photosynthetic organisms discovered by Strehler and Arnold (1951) mainly emits from photo systemII (PSII) through the inverse photochemistry reactions in oxygen evolving organisms (Strehler and Arnold, 1951; Christen et al., 2000). DF emission can in fact be induced chemically in the dark, by oxidizing the primary electron donor and reducing the primary electron acceptor (Etienne and Lavorel, 1975). DF intensity represents the integral under the decay curve and is an increasing function of the number of PSII centers, the fluorescence yield, and the rate of back reaction and it is an intrinsic fluorescence label of the efficiency of charge separation at P680 and a measure of photosynthetic activity (Schneckenburger and Schmidt, 1996; Wang et al., 2004). Investigation of DF invokes particular interest because its intensity depends directly on the rate of backward electron transport reactions in the RC of PSII. In

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its turn backward electron transport reactions are determined by quantum efficiency of primary processes of photosynthesis (Amesz and Van Gorkom, 1978; Badretdinov et al., 2004). It has been documented that there is a tight coupling between the quantum efficiencies of both PS, and that when photorespiration is suppressed there is a good correlation between the efficiency of either PS and the efficiency of CO_2 fixation (net photosynthesis rate (Pn)) (Fracheboud et al., 2002). Our previous work has revealed that there is an excellent correlation between the DF intensity and Pn in the bench study at 28 °C and normal CO_2 level ($R^2 = 0.997$) (Wang et al., 2004).

So far there are three types of methods for quantifying Pn 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-6400 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. So a steady-state Pn need about 10-min to be recorded after the leaf in the leaf chamber being irradiated (Law and Crafts-Brandner, 1999). Our previous work reported a method for measuring photosynthesis using DF of chloroplast (Wang et al., 2004). The method can accurately and fast measure the plant photosynthesis capacity under normal physiology conditions with minimal influence of the environment. But to our best knowledge, there is not such photosynthesis capacity measurement system using DF of plant leaf at present.

In the study, a novel fast and portable photosynthesis capacity biosensor was developed based on our previously reported method for measuring photosynthesis using DF. A database in the software to carry out data acquisition and processing was developed to translate maximal DF intensity to Pn. The plant photosynthesis capacity can be achieved directly *in vivo* in less than 10 s using the developed biosensor. The utility of the system was also demonstrated by contrast experiments of measuring Pn of four different plant species samples (Queen Rape Myrtle (var. rubra), soybean (Lu Hei No. 1), maize (Jin Dan No. 39) and rice (Jing Dao No. 21)) in field using the developed biosensor and commercially portable photosynthesis system LI-6400. The new principle method of photosynthesis measurement is a challenge and breakthrough to conventional method of gas exchange and may be a potential technique of next generation photosynthesis measurement.

2. Materials and methods

2.1. Theory for measurement of Pn using DF

During photosynthesis, charge separation at PSII and PSI starts due to light absorption. Electrons are transported through the electron transport chain to the Calvin cycle. Stopping illumination the processes of the light phase reverse: electrons on the electron transport chain flow back to $P680^+$, leading to $P680^*$. This $P680^*$ decays to the ground state emitting DF. The total relaxation kinetics of DF is defined as a sum of kinetic components (Lavorel, 1975):

$$I_{DF}(t) = \sum_i I_{DF_i} e^{-t/\tau_i} \quad (1)$$

where $I_{DF}(t)$ is the DF intensity at time t after illumination has ceased, I_{DF_i} the amplitude of the ith component, and τ_i is its lifetime.

DF intensity (I_{DF}) represents the integral under the decay kinetics curve (Joliot and Joliot, 1980; Monti et al., 2005):

$$I_{DF} = \int_{t=0}^{+\infty} I_{DF}(t)\,dt \quad (2)$$

I_{DF} depends directly on the rate of backward electron transport reactions in the RC of PSII. In its turn, backward electron transport reactions are determined by quantum efficiency of primary processes of photosynthesis (Amesz and Van Gorkom, 1978; Goltsev et al., 2003; Badretdinov et al., 2004). Therefore, I_{DF} can be estimated by:

$$I_{DF} \sim I_{ex} \times \varphi_{P_0} \quad (3)$$

where I_{ex} is the excitation photon energy flux density and φ_{P_0} is the quantum yield of the primary photochemical reaction in open PSII reaction centers.

The rate of PSII electron transport (r_e) in leaves under light was estimated using the following equation (Genty et al., 1989):

$$r_e = I_{ex} \times \alpha \times \varphi_{P_0} \quad (4)$$

where α was assumed to be 0.4 (Schreiber, 1994; Schreiber et al., 1998).

We can obtain by combining Eqs. (3) and (4):

$$I_{DF} \sim \frac{1}{\alpha \times r_e} \quad (5)$$

When the excitation light is saturated, Eq. (5) becomes the following formula:

$$I_{DF\,max} \sim \frac{1}{\alpha \times r_{e\,max}} \quad (6)$$

where $I_{DF\,max}$ and $r_{e\,max}$ are maximal DF intensity and the potential rate of electron transport at saturated light, respectively.

Farquhar et al. (1980) proposed that net leaf photosynthesis, Pn, could be modeled as the minimum of two limiting rates:

$$Pn = \min(P_c, P_r) \quad (7)$$

P_c is the rate of photosynthesis when Rubisco activity is limiting and P_r is the rate when ribulose-1,5-bisphosphate (RuBP)-regeneration is limiting. At low CO_2 concentration, RuBP is saturated and carboxylation of RuBP is the limiting step of photosynthesis. At high CO_2 concentration, in particular, at the optimal temperature, photosynthetic rate is limited by RuBP regeneration (Sage et al., 1990; Hikosaka et al., 2006).

Considering the above statements, we obtain Pn under the environmental conditions of saturated excitation light, optimal temperature, and saturated CO_2 concentration:

$$\mathrm{Pn} = P_r = r_{e\max}\frac{C_i - \Gamma^*}{4C_i + 8\Gamma^*} \sim \left(\frac{\alpha(C_i - \Gamma^*)}{4C_i + 8\Gamma^*}\right) \times I_{\mathrm{DF}\max} \quad (8)$$

where C_i (the intercellular concentration of CO_2), Γ^* (the *in vivo* temperature dependence of the CO_2 compensation point), and α are fixed value at the saturation. By Eq. (8), we can conclude that there is linear correlation between Pn and DF intensity under the conditions of saturated excitation light, optimal temperature, and saturated CO_2 concentration.

2.2. Culture of sample

Seeds of six soybean species (*Glycine max* (L.) Merr.) (Ke Feng No. 1, Jing Huang No. 3, Zao Shu No. 18, You Chu No. 4, Feng Jiao No. 66-12, Lu Hei No. 1), five rice species (*Oryza sativa* L.) (Shan You No. 111, Wei You No. 134, Jin You No. 207, Jing You No. 6, Jing Dao No. 21) and five maize species (*Zea May* L.) (Ji No. 853, Yun Xi No. 422, Yun Xi No. 5081, Jin Dan No. 34, Jin Dan No. 39) were first germinated on moistened filter paper at 25 °C in darkness for 2 days. All chosen plants of every species (soybean, maize, and rice) originate from the same region, which have the similar life habit and can grow well in the same environment. Seedlings were then planted into a commercial medium consisting of peat moss, vermiculite, and sand (2:1:1) in pots with 20 cm in diameter and 24 cm in height. Soybean seedlings were grown in a plant growth chamber (Conviron, model E7/2, Winnipeg, Canada) under a relative humidity (RH) of 70%/80% (day/night) and a photoperiod of 14-h with a photosynthetically active radiation (PAR) of 400 μmol photons m^{-2} s^{-1}. The growth temperature first began from 18 °C at 6 O'clock and then increased 5 °C every 2-h until the temperature reached at 33 °C at 12 O'clock. Subsequently, the plant growth temperature began from 33 °C at 14 O'clock decreasing 5 °C every 2-h until the temperature reached again at 18 °C at 20 O'clock. Then, the light was turned off and the temperature was maintained at 18 °C until 6 O'clock next morning, when the light was again turned on and the temperature was again cycled. After a week, the seedlings with uniform size were selected. Four to five soybean plants were maintained per pot. Seedlings of maize and rice were grown in an air-conditioned greenhouse (Plant Garden, South China Normal University (SCNU)) under a RH of 60%/70% (day/night) and a photoperiod of 12-h. Greenhouse plants received natural light that reached a maximum of 1800 μmol photons m^{-2} s^{-1} of PAR. The plants encountered on a temperature range of 21–36 °C and a mean temperature 30 °C lasting for about 8-h every day. Plants were watered every day and fertilized with a nutrition solution (1:500 Hyponex 5–10–5, Hyponex, Oosaka, Japan) once a week. Three Queen Rape Myrtle species (*Lagelstroemia indica* L.) (Banaba Tea, var. rubra, var. amabilis) grew in Plant Garden of SCNU. Banaba Tea and var. rubra were 1-year-old and var. amabilis 13-month-old.

2.3. Measurement of Pn

Pn was determined on the most recent fully expanded leaves of 1-year-old Queen Rape Myrtle, 9-week-old soybean, 8-week-old rice, and 10-week-old maize with a portable photosynthesis system LI-6400 (Li-Cor, Lincoln, NE, USA) using a built-in light source set at the indicated PAR in the morning (9:30–12:30). The measurements were performed using the 11 leaves chosen at random. Pn was obtained at the indicated optimum temperature and saturated concentration of CO_2. Steady-state Pn was recorded after the leaf in the leaf chamber being irradiated about 10-min (Law and Crafts-Brandner, 1999).

2.4. Biosensor system

2.4.1. Concept of operation

Two different operation modes (remote and local control) were optional. In the remote control, the operation of the main instrument was carried out through a personal computer (PC), while in the local mode, the operation was accomplished on the front panel of the instrument. At first parameters including light excitation intensity, excitation time, sample interval, the experimental duration, CO_2 concentration, RH, and temperature were set. Then according to concrete parameters, drive the excitation light source to irradiate the sample for the set excitation time, stop the light, sample one time every interval until reaching the experimental duration. The measurement process of DF signal was divided into two steps: background survey and meterage of mix signal including DF and background. DF decay dynamics curve was obtained by subtracting background from mix signal. In the biosensor, since the DF signal was stable at the 0.26 s and decreased to nearly zero at the 5.26 s, DF intensity was integrated from 0.26 to 5.26 s under DF decay dynamics curve. Finally, through the establishment of a correlation database between maximal DF intensity and corresponding maximal Pn, maximal DF intensity was converted to corresponding maximal Pn (the photosynthesis capability), which displayed as a number on a display (local control mode) and PC screen (remote control mode) in terms of Pn.

2.4.2. System hardware design

The major hardware system block diagram of the biosensor is shown in Fig. 1. The system was mainly composed of the following hardware parts: dark sample chamber, exciting light source, CO_2 and humidity controller, temperature controller, Channel Photomultiplier DC-Module (CPDM (MD963, Perkin-Elmer, Wiesbaden, Germany)), and data acquisition and processing system. The super high light light-emitting diode (LED) lattice was used as excitation light source, which comprised six LEDs, whose center wavelength was 628 nm, half-wave width 20 nm, single duct output luminous flux 20 lm and the excitation light intensity at the sample could reach 2500 μmol photons m^{-2} s^{-1}. CPDM was adopted to receive the DF signal, whose output range was 0–10 V, detection wavelength range 185–850 nm. The cutting filter of 660 nm was designed

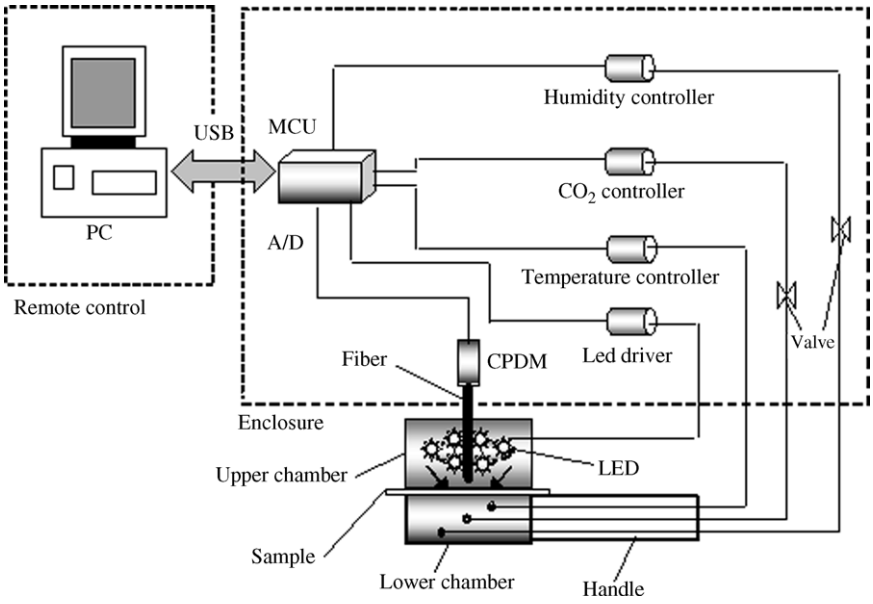

Fig. 1. DF-based photosynthesis capacity biosensor block diagram illustrating major hardware subsystems. Power supplies omitted for sake of clarity.

to protect CPDM from damage of excitation light irradiation. The CO_2 concentration can be adjusted from 0 to 2000 ppm and the precision was ±40 ppm, and the humidity was controlled from 5 to 95%. The temperature control range was from 15 to 40 °C and precision can reach 0.01 °C. Data acquisition and processing was accomplished by micro control unit (MCU (AT89c55)) in the local control mode and PC in the remote control mode.

2.4.3. Dark sample chamber

The structure of dark sample chamber is described in Fig. 2. The dark chamber included upper and lower chambers, which were folio up and down and connected by gemel. The protuberant ring and groove were designed in the upper and lower chambers, respectively, which combined to form the dark cavity. In order to place live plant leaf conveniently, a blowhole was devised with black hermetic foam cushion, which was used to tighten the leaf stalk and shelter from the outside light. The lock and open of the dark sample chamber was accomplished by a lock and spring. There were three sensors (temperature sensor, humidity sensor and CO_2 sensor) in the lower chamber (not shown in Fig. 2).

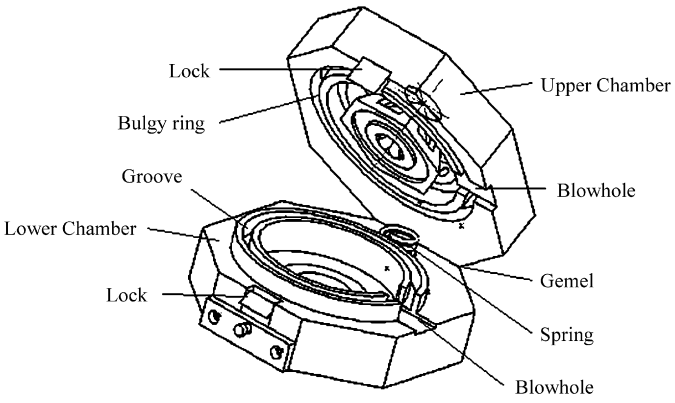

Fig. 2. The structure of dark sample chamber.

2.4.4. LED drive

To stabilize the light intensity of LED, a low-noise constant current source at the output level was adopted, which can stabilize the current with little ripple coefficient. LED as the load is in series with high-power Field Effect Transistor, which was used to drive LED. Auto current control current feedback technique was used to stabilize positive drive current and realize continuing regulation in the range of 0–100 mA and the control precision is 0.1 mA.

2.4.5. CO_2 and humidity control

Non-dispersive infrared detector and HS series capacitor sensor were used as the inductor for CO_2 concentration and humidity, respectively. CO_2 steel bottle and water box were connected separately with the dark sample chamber by two plastic and hermetic pipes, on which two valves were installed. The valves were controlled by AT89c55 to open when the actual value was less than the set value and close when the actual value was equivalent to the set value. The CO_2 concentration can be adjusted in the range from 0 to 2000 ppm and the precision was ±40 ppm and the humidity was controlled in the range from 5 to 95%.

2.4.6. Temperature control

The difference between the environmental temperature and the set drove the executant (thermal energy converter (TEC)) to work to stabilize the environmental temperature at the set temperature. When working, TEC can stabilize the temperature through refrigerating one side and heating the other side by changing current direction. Proportion integral control was utilized to reduce static state error and improve control precision.

In the system, the temperature control range is 15–40 °C and precision can reach 0.01 °C.

3. Results and discussion

3.1. Optimization of influencing parameters on DF intensity

As stated in above part of theory for measurement of Pn using DF, there was linear correlation between DF intensity and Pn under the conditions of saturated excitation light, optimal temperature, and saturated CO_2 concentration. However these parameters may be different for the different plants. Therefore, the confirmation of these optimal parameters had to be performed to obtain the maximal DF intensity, and hence Pn. Further, in order to make the optimization process simple, a fixed CO_2 concentration (1250–1350 ppm) was chosen, which was saturated for C_3 and C_4 plants (Cheng et al., 2001). In addition, DF intensity was also affected by the excitation time and excitation intensity. Here take the leaves of soybean (Jing Huang No. 3) as the model samples to illustrate this optimization process of parameters (excitation time, excitation intensity, and temperature) to obtain maximal DF intensity. Similar experiments were performed for the leaves of other five soybean species, five rice species, five maize species and three Queen Crape Myrtle species. The experimental results were summarized in Table 1.

3.1.1. Effect of the excitation time on the DF intensity

By setting the LED light intensity at 1000 μmol photons m^{-2} s^{-1}, the DF intensity as a function of light excitation time was studied. DF intensity was maximum in the range of 200–1000 ms. When the excitation prolonged, the DF intensity gradually degraded, probably caused by photoinhibition, which was defined as the decrease in photosynthetic activity upon excess illumination (Velitchkova and Picorel, 2004). Based on the investigation, the LED light intensity in the range from 200 to 1000 ms at 1000 μmol photons m^{-2} s^{-1} was used to measure DF.

3.1.2. DF intensity response to the excitation intensity

The relationship between intensities of the LED excitation light and the DF intensity was investigated. The excitation time was set at 200 ms. The DF intensity was linearly correlated to the excitation light intensity when the irradiation intensity is less than 1000 μmol photons m^{-2} s^{-1}. As the irradiation intensity increased above 1000 μmol photons m^{-2} s^{-1}, DF signal gradually reached saturation. Further increase in the irradiation intensity had even negative effect on DF intensity, which may be caused by photoinhibition. Maximum and stable DF signal can be obtained by setting the irradiation light intensity at the saturation point. It should be noted that the total excitation energy required to obtain maximum DF was different for each plant. For soybean (Jing Huang No. 3), the DF saturation was reached at 1000 μmol photons m^{-2} s^{-1} (the excitation time was 200 ms).

3.1.3. Response of DF intensity to temperature

The relationship between sample temperature and the intensity of DF was also researched. Maximum DF intensity was observed at 26 °C. The optimal temperature to reach maximum DF varied for different plants. The dependence of DF intensity on temperature is nearly identical to that of the photosynthesis rate (Shen, 2000b). The correlation between DF intensity and Pn across a range of temperatures and the mechanisms involved has been studied in our lab.

Table 1
Optimal parameters to obtain maximal DF intensity

Plant species	Excitation time (ms)	Excitation intensity (μmol m^{-2} s^{-1})	Temperature (°C)
Soybean			
Ke Feng No. 1	200	950	27
Zao Shu No. 18	200	1100	26.5
You Chu No. 4	190	1000	25.5
Feng Jiao No. 66-12	210	900	28
Lu Hei No. 1	220	950	26
Rice			
Shan You No. 111	210	1000	28
Wei You No. 134	220	1200	27
Jin You No. 207	230	1000	25.5
Jing You No. 6	210	1300	28
Jing Dao No. 21	200	1100	26.5
Maize			
Ji No. 853	230	1500	28
Yun Xi No. 422	240	1600	30
Yun Xi No. 5081	220	1600	29
Jin Dan No. 34	240	1700	31
Jin Dan No. 39	240	1500	30
Queen Crape Myrtle			
Banaba Tea	210	800	27
Var. rubra	200	950	28
Var. amabilis	200	850	28.5

3.2. The database of correlation between maximal DF intensity and Pn

The establishment of a correlation database between maximal DF intensity and corresponding maximum Pn was necessary in order to obtain Pn based on DF intensity in the lab-built biosensor. The correlation of DF intensity and Pn was achieved by the contrast experiment of DF intensity and Pn under the optimal conditions. Take the leaves of soybean (Jing Huang No. 3) as the model samples to illustrate this process in detail. The leaf samples of soybean (Jing Huang No. 3) were studied in the controlled climate chamber (excitation intensity, excitation time and temperature are indicated in Table 1. RH is 75% and CO_2 concentration 1250–1350 ppm). Both DF system and the commercial unit were set up in the identical ways. The results are shown in Fig. 3. Statistical analysis showed that there was an excellent linear correlation between the DF intensity and Pn under the conditions of saturated excitation light, optimal temperature, and CO_2 concentration ($R^2 = 0.988$, Fig. 3). The statistical result was accordant with that in the part of theory for measurement of Pn using DF.

The correlation between maximal DF intensity and Pn can be expressed using the following formula:

$$\mathrm{Pn} = \frac{I_{\mathrm{DF\,max}} - A}{B} \tag{9}$$

where A and B are the intercept of $I_{\mathrm{DF\,max}}$ axis and slope of fit line, respectively.

In the experiment, $A = 37.7 \pm 5.4$ and $B = 8.1 \pm 0.5$, substituting in Eq. (9):

$$\mathrm{Pn} = 0.1 \times (I_{\mathrm{DF\,max}} - 37.7) \tag{10}$$

Eq. (10) shows the arithmetical relation between maximal DF intensity and Pn of soybean (Jing Huang No. 3), which is used as a mathematical model of soybean (Jing Huang No. 3) database of relation between maximal DF intensity and Pn. Similar experiments were performed for the leaves of other five soybean species, five rice species, five maize species and three Queen Crape Myrtle species. These results also show good linear correlation between the DF intensity and Pn under optimal conditions. The corresponding results of statistical relations are shown in Table 2.

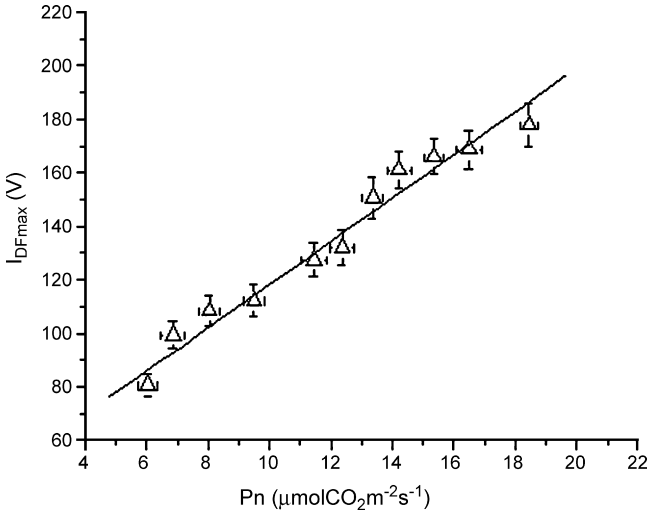

Fig. 3. Correlation between maximal DF intensity and Pn of soybean (Jing Huang No. 3). Experimental conditions: excitation light intensity, 1000 μmol photons m^{-2} s^{-1}; excitation time, 200 ms; temperature, 26 °C; concentration of CO_2, 670–720 ppm; RH, 75%. Data are the mean ± S.E. of five replicates.

Table 2
Mathematical model of relation between maximal DF intensity and Pn

Plant species	Pn (μmol m^{-2} s^{-1})
Soybean	
Ke Feng No. 1	$0.2 \times (I_{\mathrm{DF\,max}} - 31.5)$
Zao Shu No. 18	$0.2 \times (I_{\mathrm{DF\,max}} - 35.7)$
You Chu No. 4	$0.2 \times (I_{\mathrm{DF\,max}} - 39.4)$
Feng Jiao No. 66-12	$0.2 \times (I_{\mathrm{DF\,max}} - 37.9)$
Lu Hei No. 1	$0.2 \times (I_{\mathrm{DF\,max}} - 42.7)$
Rice	
Shan You No. 111	$0.2 \times (I_{\mathrm{DF\,max}} - 41.8)$
Wei You No. 134	$0.2 \times (I_{\mathrm{DF\,max}} - 39.3)$
Jin You No. 207	$0.2 \times (I_{\mathrm{DF\,max}} - 49.2)$
Jing You No. 6	$0.2 \times (I_{\mathrm{DF\,max}} - 53.2)$
Jing Dao No. 21	$0.2 \times (I_{\mathrm{DF\,max}} - 47.5)$
Maize	
Ji No. 853	$0.2 \times (I_{\mathrm{DF\,max}} - 24.6)$
Yun Xi No. 422	$0.3 \times (I_{\mathrm{DF\,max}} - 29.5)$
Yun Xi No. 5081	$0.3 \times (I_{\mathrm{DF\,max}} - 34.5)$
Jin Dan No. 34	$0.3 \times (I_{\mathrm{DF\,max}} - 30.7)$
Jin Dan No. 39	$0.2 \times (I_{\mathrm{DF\,max}} - 28.9)$
Queen Crape Myrtle	
Banaba Tea	$0.1 \times (I_{\mathrm{DF\,max}} - 37.1)$
Var. rubra	$0.1 \times (I_{\mathrm{DF\,max}} - 42.6)$
Var. amabilis	$0.1 \times (I_{\mathrm{DF\,max}} - 34.3)$

3.3. Field testing

To demonstrate the ability of the developed biosensor, field contrast experiments for measuring Pn of plant leaf using the biosensor and commercial photosynthesis system LI-6400 were performed for four different plant species: Queen Rape Myrtle (var. rubra, 1-year-old, grown in the South China Botanical Garden, Chinese Academy of Sciences), soybean (Lu Hei No. 1, 10-week-old), maize (Jin Dan No. 39, 9-week-old) and rice (Jing Dao No. 21, 8-week-old). The latter three crops are all grown in the plant garden of South China Agriculture University. Excitation intensity, excitation time and temperature are shown in Table 1. RH is 75% and CO_2 concentration 1250–1350 ppm. The biosensor was set in the same ways as the commercial unit. The results are shown in Fig. 4. The Pn from the biosensor exhibited good agreement with that from the commercial LI-6400 for different plant species. The consistency comes from good linear correlation between Pn and DF intensity at the saturated excitation light, optimal temperature, and CO_2 concentration. Statistical analysis showed that uncertainty of the results using two instruments was less than ±5%, which is acceptable. Based on the results we can conclude that the biosensor was reliable and precise. In addition, compared with LI-6400, the statistical results showed that measurement time using the biosensor is less

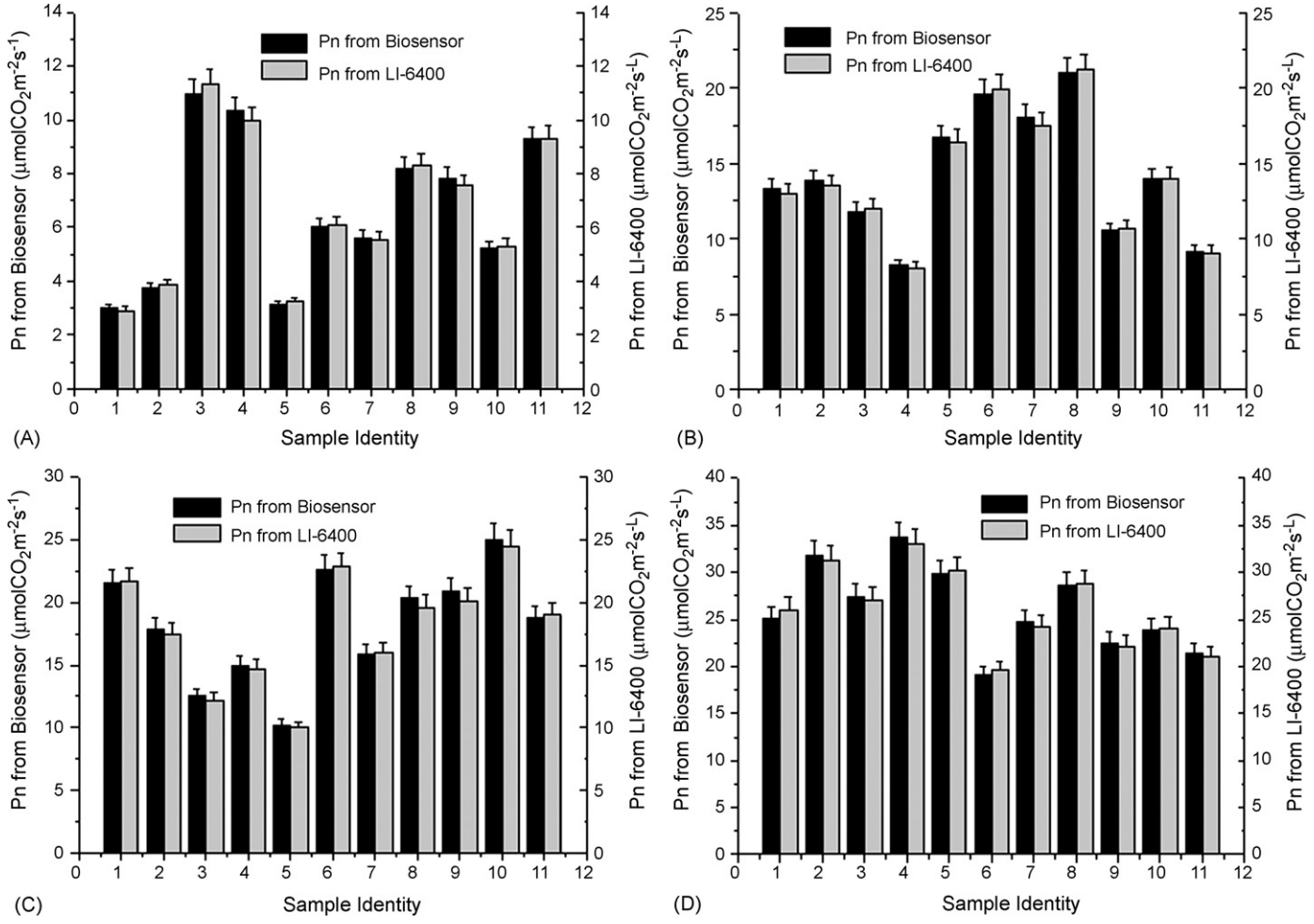

Fig. 4. Contrast measurement of Pn using biosensor and LI-6400 at the same conditions. (A) Queen Rape Myrtle (var. rubra) (excitation light intensity, 950 μmol photons m^{-2} s^{-1}; excitation time, 200 ms; temperature, 28 °C). (B) Rice (Jing Dao No. 21) (excitation light intensity, 1100 μmol photons m^{-2} s^{-1}; excitation time, 200 ms; temperature, 26.5 °C). (C) Soybean (Lu Hei No. 1) (excitation light intensity, 950 μmol photons m^{-2} s^{-1}; excitation time, 200 ms; temperature, 26 °C). (D) Maize (Jin Dan No. 39) (excitation light intensity, 1500 μmol photons m^{-2} s^{-1}; excitation time, 240 ms; temperature, 30 °C). Concentration of CO_2, 1250–1350 ppm; RH, 75%. Data are the mean ± S.E. of five replicates.

than 10 s, while a steady Pn value got by LI-6400 needs about 5–10 min. Furthermore, the developed biosensor with remote control has the predominance of low cost, simple and convenient operation, and original and compact appearance. These merits would make the developed biosensor have the powerful competition in fast, real-time examination and longtime inspection of plant growth and development and precision agriculture. The new principle of photosynthesis measurement is a challenge and breakthrough to conventional method of gas exchange and it may be a potential technique of next generation photosynthesis measurement.

4. Conclusions

In this study, a portable photosynthesis capacity biosensor based on quantitative measurement of DF *in vivo* was developed. The novel features of the biosensor described here include: (1) the biosensor was developed based on a new principle—measuring photosynthesis capacity using DF of plant leaf, resulting in less interference from environment than conventional method for measuring Pn based on CO_2 consumption. This new method is a challenge and breakthrough to conventional method of gas exchange and it may be a potential technique of next generation photosynthesis measurement. (2) The biosensor has characteristics of rapidity (the measurement time is less than 10 s, while a steady Pn value got by LI-6400 need about 5–10 min). (3) The biosensor has the merits of low cost, simple and convenient operation, and remote control, which would make it have the powerful competition in fast, real-time examination and longtime inspection of plant growth and development and wide application in precision agriculture. (4) The biosensor is portable because of the use of CPDM and MCU technique. (5) The biosensor realized the locale survey *in vivo* owing to the use of hermetic dark sample chamber and battery power supply. (6) The biosensor accomplished maximum Pn measurement based on a relationship database between maximal DF intensity and corresponding maximum Pn, which was developed in the data acquisition and processing software. In addition, a straightforward combination of DF intensity and photosynthesis processes made the biosensor possible to be applied

in other fields, for example, examination of environment as acid rain, salt, alkali and heat et al, quality evaluation of fruits and vegetables, electron transport efficiency at PSII and so on.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2006.12.007.

References

Amesz, J., Van Gorkom, H.J., 1978. Plant Physiol. 29, 47–66.
Badretdinov, D.Z., Baranova, E.A., Kukushkin, A.K., 2004. Bioelectrochemistry 63, 67–71.
Cheng, L.L., Fuchigami, L.H., Breen, P.J., 2001. J. Exp. Bot. 52, 1865–1872.
Christen, G., Steffen, R., Renger, G., 2000. FEBS Lett. 475, 103–106.
Etienne, A.L., Lavorel, J., 1975. FEBS Lett. 57, 276–279.
Farquhar, G.D., Von Caemmerer, S., Berry, J.A., 1980. Planta 149, 78–90.
Fracheboud, Y., Ribaut, J.M., Vargas, M., Messmer, R., Stamp, P., 2002. J. Exp. Bot. 53, 1967–1977.
Genty, B., Briantais, J.M., Baker, N.R., 1989. Biochim. Biophys. Acta 990, 87–92.
Goltsev, V., Zaharieva, I., Lambrev, P., Yordanov, I., Strasser, R., 2003. J. Theor. Biol. 225, 171–183.
Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., Onoda, Y., 2006. J. Exp. Bot. 57, 291–302.
Joliot, P., Joliot, A., 1980. Plant Physiol. 65, 691–696.
Khuznetsova, S., Bogracheva, T., Hedley, C., Tuleshova, A., Kukushkin, A., 2001. Ann. Appl. Biol. 138, 117–121.
Lavorel, J., 1975. In: Govindjee (Ed.), Bioenergetics of Photosynthesis. Academic Press, New York, pp. 223–317.
Law, R.D., Crafts-Brandner, S.J., 1999. Plant Physiol. 120, 173–181.
Monti, M., Zrimec, A., Beran, A., Zrimec, M.B., Drinovec, L., Kosi, G., Tamberlich, F., 2005. Harmful Algae 4, 643–650.
Rutherford, A., Inoue, Y., 1984. FEBS Lett. 165, 163–170.
Sage, R.F., Sharkey, T.D., Pearcy, R.W., 1990. Aust. J. Plant Physiol. 17, 135–148.
Sane, P., Rutherford, A., 1986. Light Emission by Plants and Bacteria. Academic Press, New York, pp. 329–360.
Schneckenburger, H., Schmidt, W., 1996. J. Plant Physiol. 148, 593–598.
Schreiber, U., 1994. Z. Naturforsch. 49, 646–656.
Schreiber, U., Bilger, W., Hormann, H., Neubauer, C., 1998. In: Raghavendra, A.S. (Ed.), Photosynthesis. A Comprehensive Treatise. Cambridge University Press, Cambridge, pp. 320–336.
Shen, Y.G., 2000a. The Important Chemistry Reaction on Earth Photosynthesis. QinHua University Press, Beijing, pp. 84–85.
Shen, Y.G., 2000b. The Important Chemistry Reaction on Earth Photosynthesis. QinHua University Press, Beijing, pp. 86–89.
Strehler, B., Arnold, W., 1951. J. Gen. Physiol. 34, 809–820.
Velitchkova, M.Y., Picorel, R., 2004. Biophys. Chem. 107, 25–32.
Wang, C.L., Xing, D., Chen, Q., 2004. Biosens. Bioelectron. 20, 454–459.