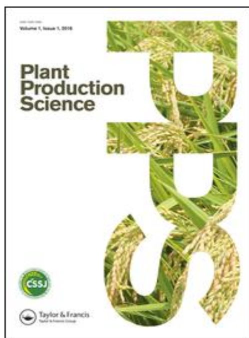

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[Short Report]

Concurrent Monitoring of Oxygen Evolution and Chlorophyll Fluorescence in Mungbean Leaves with a Liquid-Phase Oxygen Electrode

Yasuyuki Yoshimura*, Kenji Hirao and Fumitake Kubota

(**Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University; Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan*)

Key words: Chlorophyll fluorescence, Oxygen electrode, *Vigna radiata* (L.).

The liquid-phase oxygen electrode is widely used for determining photosynthetic rate by monitoring oxygen evolution from a leaf placed in solution (Ishihara et al., 1979; Ishii et al., 1977). If it is possible to make measurement of the emittance of chlorophyll fluorescence from a leaf concurently with oxygen evolution by the electrode method, it may become a useful technique for studying the energy flow in photosynthesis of leaf in various solutions. However, fluorescence can not be determined accurately by the conventional method in which sliced small leaf pieces are stirred in a CO_2-saturated solution. Stirring promotes CO_2 transport from the solution to leaf mesophyll, but the fluorescence can not be detected correctly from moving objectives. Technical improvement is necessary to measure oxygen evolution rate (OER) and fluorescence concurrently.

Yatomi et al. (1992) demonstrated that OER was correctly measured using a nonsliced leaf with the abaxial epidermis peeled off, because the barrier of CO_2 transport between the solution and mesophyll was removed by peeling the epidermis. Based on this finding, we set up a system for concurrent measurement of oxygen evolution and fluorescence by remodeling a conventional type of liquid-phase oxygen electrode, and applied it for measurements of mungbean leaves.

Material and Methods

1. Measurements

Expanded leaves of mungbean, *Vigna radiata* (L.) Wilczek, var. Chinese, were used.

Fig. 1 shows the improved system of a liquid-phase oxygen electrode (Rank Brothers Engineering, U.K.) combined with a portable fluorometer (PAM-2000, Walz, Germany). A ring-shaped leaf holder was made of plastic resin and placed in the reaction cup to fix a leaf disc with the abaxial epidermis peeled off (peeled leaf disc, PLD) in the stirring solution. PLD was 10 mm in diameter. The fluorescence detector rod was placed inside of the cap cylinder, and light was vertically irradiated through the rod to the surface of PLD. The values measured with PLD were compared with those obtained by the conventional method using sliced leaf pieces (SLP).

PLD or SLP, each of which was vacuum-infiltrated in buffer, was placed in the reaction cup filled with 4 mL of 50 mM HEPES solution (pH 7.2) containing 0.5 mM $CaSO_4$ and irradiated at a photosynthetically active photon flux density (PPFD) of 640 $\mu mol\ m^{-2}\ s^{-1}$. After 20 min of irradiation, photosynthesis was allowed to start by injecting 100 μl of 0.833 M $NaHCO_3$ into the reaction cup with a micro-syringe. The solution was stirred with a magnetic stirrer and maintained at a temperature of 25 or 30°C. OER and fluorescence were concurrently measured at 60 s intervals. The quantum yield (Φ_{PSII}) at photosystemII was calculated from chlorophyll fluorescence quenching.

2. Calculation of Φ_{PSII} and the number of electrons (k)

Φ_{PSII} was calculated from eq. (1) proposed by Genty et al. (1989).

$$\Phi_{PSII} = (Fm' - Fs)/Fm' \qquad (1)$$

where Fs was the chlorophyll-fluorescence emittance of leaf measured after the oxygen evolution rate came to be stable, and Fm' was the fluorescence peak shown by giving 1.2 s pulse of saturation light.

The parameter k is the number of electron equivalents required for 1 mol O_2 evolution or 1 mol CO_2 reduction, and calculated from eq. (2).

$$k = \Phi_{PSII} \times L \times 0.5 \times a/OERg \qquad (2)$$

where L is the photon flux density of leaf surface (PPFD of 640 $\mu mol\ m^{-2}\ s^{-1}$). Assuming the even distribution of photons to the two photosystems, 0.5 is added to eq. (2). Parameter a is the ratio of chlorophyll-absorbed photons to the incident photons; a=0.935, which was determined with a mungbean leaf, is used here. OERg, the gross oxygen evolution rate, is the value of respiratory oxygen uptake rate plus photosynthetic oxygen evolution rate.

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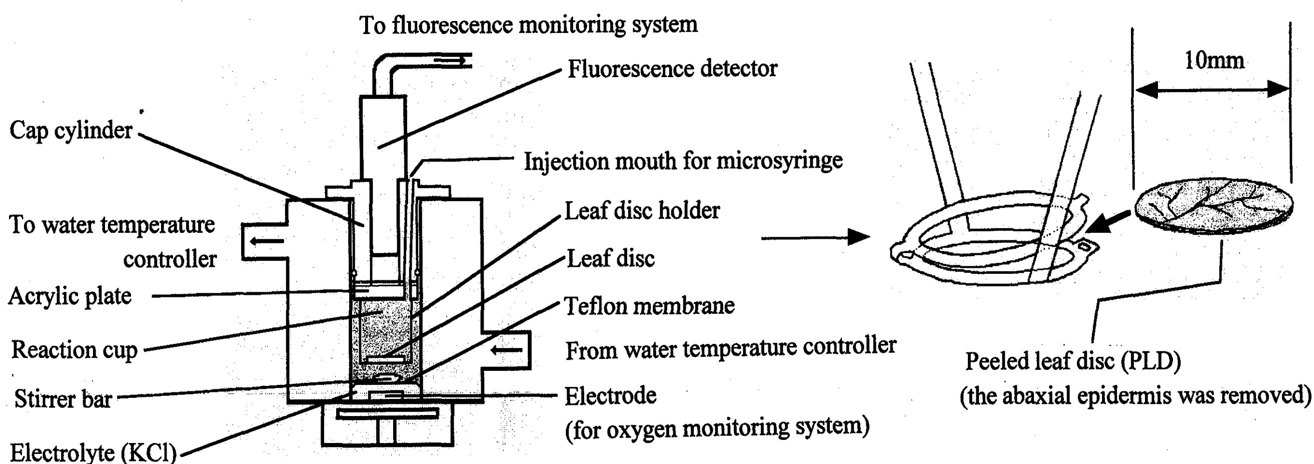

Fig. 1. The improved liquid-phase oxygen electrode system for concurrent measurement of OER and fluorescence. The clearance between the fluorecence detector and the leaf surface was 20±2 mm.

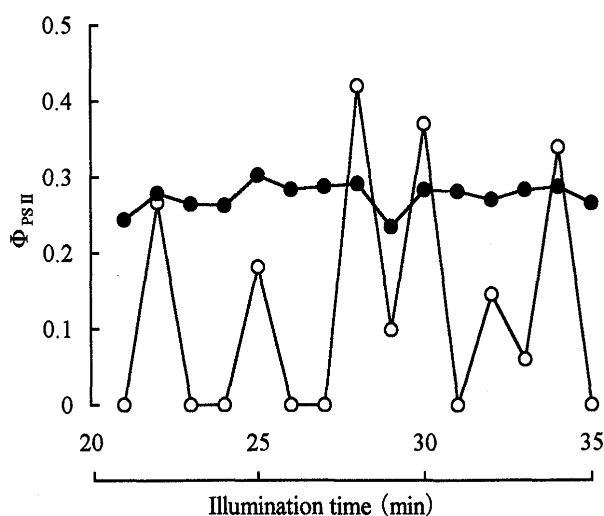

Fig. 2. Comparison of Φ_{PSII} measured with sliced leaf pieces (SLP, ○) by the conventional method and that measured with peeled leaf discs (PLD, ●) by the remodeled system shown in Fig. 1.

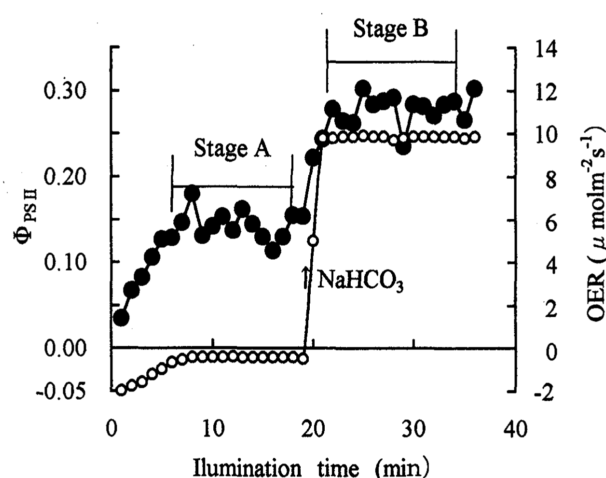

Fig. 3. Effects of $NaHCO_3$ application on Φ_{PSII} (●) and oxygen evolution rate (○) in peeled leaf disc (PLD). Average value of k was 28.8 at Stage A and 7.16 at Stage B.

Results and Discussion

The Φ_{PSII} of PLD and SLP calculated from eq. (1) are shown in Fig. 2. The Φ_{PSII} of SLP fluctuated in a wide range, but the values measured with PLD were almost constant, which made an accurate determination possible.

Fig. 3 represents the time courses of OER and Φ_{PSII} of PLD monitored at 25°C under a PPFD of 640 μmol m^{-2} s^{-1}. Φ_{PSII} increased linearly with time at the initial phase and reached a stable level (stage A). OER at this stage read almost zero because photosynthetic CO_2 intake was counterbalanced with respiratory CO_2 evolution, and the value of k calculated from eq. (2) was 28.8.

Immediately after $NaHCO_3$ was injected into the reaction solution, Φ_{PSII} and OER began to increase in parallel and reached saturation (stage B), showing average values of 0.28 and 9.86 μmol m^{-2}s^{-1}, respectively (Fig. 3). On the other hand, the value of k decreased to 7.16 at stage B, which corresponded to 24.9% of that (28.8) determined at stage A.

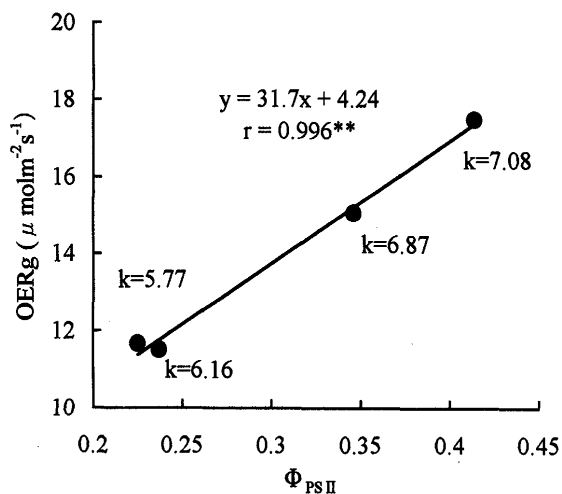

Fig. 4. The relationship between OERg (●) and Φ_{PSII} in peeled leaf disc (PLD). **, significant at 1.0% level.

A significantly positive correlation was found between OER and Φ_{PSII} measured with PLDs of different ages (Fig. 4). The values of k, Φ_{PSII} and OER were lower in aged leaves. The values of k ranged from 5.77 to 7.08, which seems to be similar to the values usually observed in C_3 crop leaves with fully opened stomata in the

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atmospheric air (Hirao et al., 1999). This evidence may support the methodological correctness of the measurement of chlorophyll fluorescence.

It may be predicted that photorespiration would be almost completely suppressed in the liquid-phase oxygen electrode method because the reaction solution was saturated with CO_2 in concentration. Contrary to the expectation, the values of k obtained here (5.77~7.08) was considerably larger than the theoretically predicted value obtained without photorespiration ($k=4$) reported by Krall and Edward (1992). This suggests that photorespiration was not sufficiently restricted here. The considerably lower diffusion efficiency of CO_2 in solution compared to that in the air is one of the reasons for the occurrence of photorespiration in leaf placed in the CO_2-saturated solution.

The concurrent monitoring of photosynthesis and chlorophyll fluorescence has been reported by Delieu and Walker (1983), Tyystjarvi et al. (1998) and others, but no or little information has been obtained on the concurrent measurement in leaves using a liquid-phase oxygen electrode. The concurrent measurement was hindered by the technical difficulty in obtaining stable and accurate values of fluorescence emittance. As mentioned above, by partly improving the conventional oxygen electrode method, the concurrent measurements of fluorescence and OER have been possible and expected to be widely applicative to the photosynthetic diagnosis of crop leaves.

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