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Concurrent Monitoring of Oxygen Evolution and Chlorophyll Fluorescence in Peeled Leaves with a Liquid-phase Oxygen Electrode

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Introduction

In the liquid-phase oxygen electrode, photosynthetic rate is determined by the rate of oxygen evolution from sliced leaf pieces stirred in CO_2-saturated reaction solution. The slicing and stirring treatments promote CO_2 absorption of leaf pieces, but there is some difficulty in determining fluorescence emittance from the moving objectives. The barrier of CO_2 uptake into leaf was removed by peeling its epidermis, and oxygen evolution rates from peeled leaves were readily measured (Yatomi, M. et al. 1992). A liquid-phase oxygen electrode was partly improved to make concurrent measurements of oxygen evolution rate and chlorophyll fluorescence quenching. In this study, we used this tool and observe the responses of gross oxygen evolution rate (Og), the quantum yield of PSII (Φe) and photorespiration rate (Pr) to the changes of photosynthetic environment.

Material and Method

As the experimental material, leaves of mungbean (*Vigna radiata* (L.) Wilczek; Cultivar, Chinese) were used. Fig.1 shows a diagram of the measurement system of oxygen evolution rate and chlorophyll fluorescence quenching. The system was set up with a liquid-phase oxygen electrode (Rank Brothers Engineering, England) and a portable fluorometer (PAM-2000, Walz, Germany). A ring-shaped holder was equipped in the reaction cup to fix a peeled leaf disc (0.785cm^2) in the reaction solution. A detector rod of the fluorometer was inserted into and fixed in the cap of the oxygen electrode. Light was vertically illuminated through the rod to the peeled leaf disc surface. The reaction cup was filled with 3ml of 50mM HEPES solution (pH 7.2) containing 0.5mM $CaSO_4$. The reaction solution was stirred with a magnetic stirrer, and the solution temperature was maintained at 30 ºC.

Φe was calculated from the equation 1 demonstrated by Genty et al. (1989).

$$\Phi e = Fm'-Fs/Fm' \qquad (1)$$

where Fs was a fluorescence emittance measured after the oxygen evolution rate of a peeled leaf disc reach a constant level and Fm' was a fluorescence peak measured by giving 1.2 sec pulse of saturation light.

The leaf disc was vacuum-infiltrated in buffer in advance, and placed in the reaction cup under illumination. After 20 minutes, photosynthesis was allowed to begin by injecting 100 μl of 0.833M $NaHCO_3$ into the reaction cup with a microsyringe. Og (respiratory oxygen uptake rate + photosynthetic oxygen evolution rate) and Φe of a peeled leaf disc were measured at 60-second intervals.

At first, we examined the effects of osmotic potential in the reaction solution on oxygen evolution rate. The osmotic potential of the reaction solution was varied in the range -0.3 to -2.0MPa by adding mannitol or NaCl.
When Og and Φe came to a constant after $NaHCO_3$ was added into the reaction solution, aminoacetonitrile (ANN), an inhibitor of photorespiration, was added into the solution (final concentration 48mM). Pr was estimated from the equation 2.

$Pr = O_\Phi - Og$ (2)

where O_Φ is the total oxygen evolution rate (O_Φ).

O_Φ was calculated from the equation 3.

$O_\Phi = \Phi e \times 0.5 \times iL/4$ (3)

where L is a light intensity supplied to a peeled leaf disc. Assuming the photon is distributed even to the two photosystems, 0.5 is used in the equation 3. *i* is the ratio of the photon absorbed by leaf to the incident photon; *i* = 0.8 is used here. The number of 4 is the equivalent number of electrons used for the evolution of 1 O_2 at PSII.

After Og and Φe came to a constant, ATP (1M) was added to the reaction solution and the time courses of changes in Og and Φe were monitored.

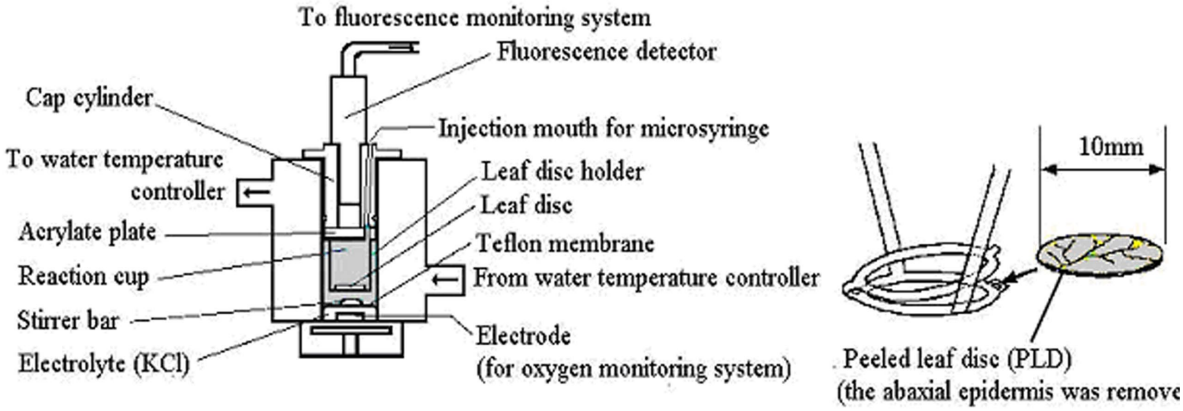

Fig. 1. The improved liquid-phase oxygen electrode system for concurrent measurement of oxygen evolution rate and chlorophyll fluorescence quenching.

Results and Discussion

Both Φe and oxygen evolution rate decreased, as osmotic potential of the reaction solution was reduced by NaCl or mannitol addings. A reduction in osmotic potential from -0.3 to -1.5 MPa did not affect Og, but Og reduced below -1.5MPa. Also, Φe was constant at the level -0.3 to -1.0 MPa , but gradually decreased below -1.0MPa (Fig.2).

In the case of NaCl addition, it may be considered that the responses are affected by two causes such as osmotic potential change and Na^+ or/and Cl^- ions. However, the ionic effects on Og and Φe were not found here.

As shown in Fig. 3, the ratio of Ogr/er was almost unchanged in the solution osmotic potential from -0.3 to -1.0MPa (Stage 1), and then increased with a decrease in osmotic potential to -1.5MPa (Stage 2). Below -1.5MPa, the ratio was at a plateau again (Stage 3). At the stage 1 the solution osmotic potential did not affect both Og and e, but at the stage 2 to 3, the ratio of Og (the energy consumption) to Φe (the energy production) increased by the

decrease in e. Because Og was constant at these stages except for the lowest osmotic potential, the increased ratio means that the produced chemical energy production was effectively used in the high osmotic potential solution.

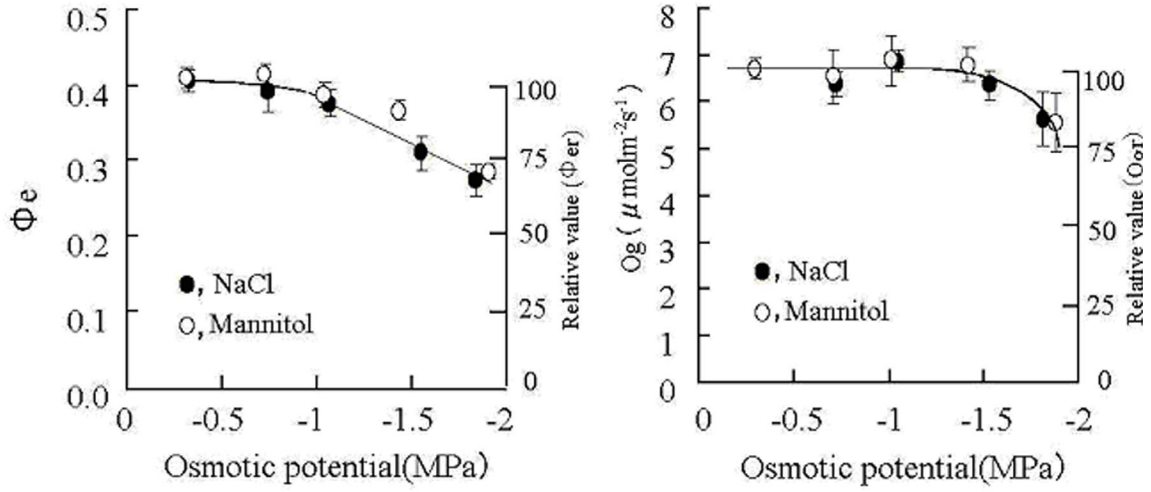

Fig.2 The effects of osmotic potential of the reaction solution on Φ_e and Og.
Values are means ± standard errors of 4 replicates.

The addition of ANN, an inhibitor of photorespiration, into the reaction solution decreased not only Pr but also Og, whereas the Pr ratio, $(O_\Phi - Og)/ O_\Phi$, was unchanged (Fig.4).

Immediately after ATP addition into the reaction solution, both Og and Φe showed a temporary depression, and recover to their previous levels about 10 minutes later (Data is not shown here).

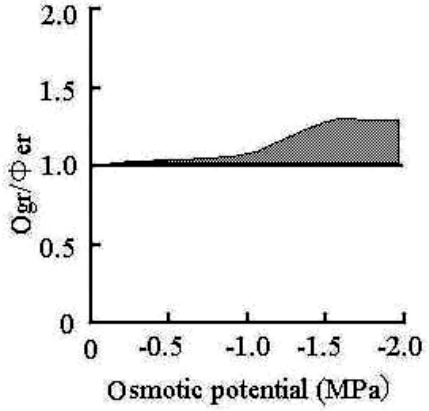

Fig. 3 Changes in Ogr/Φer with osmotic potential in the reaction solution. The data were derived from Fig.2.

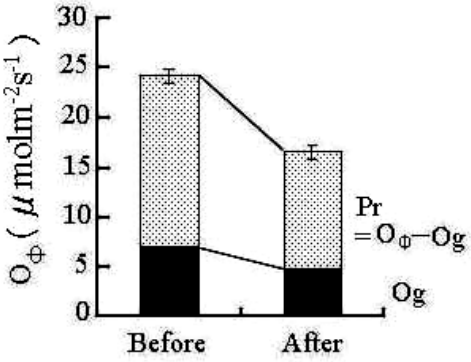

Fig.4 The effects of ANN on gross O_2 evolution rate (■) and photorespiratory rate (▨). Before, measured before ANN addition ; After, measured after ANN addition . Values are means standard errors of 4 replicates.

Conclusion: By applying the remodeled oxygen electrode examined here to survey and evaluation of the photosynthetic characteristics of many crop species and varieties, we can make a more detailed analyses and discussion on their responses to different environmental conditions from the two view points of chemical energy production and consumption.

References

Genty, B., J –M. Briantais and N. Baker (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta.* 990. 87-92

Yatomi, M., F. Kubota, W. Agata and M. Morokuma (1992) Measurement of photosynthetic rate in Sweet Potato (*Ipomoea batatas Lam.*) leaf by oxygen electrode method. *Jpn. J. Crop Sci*. 61. 312-313