Genetic Diversity and Inheritance of Photosynthetic Capacity of Rice *Oryza sativa* L. Detected by Oxygen Evolution in Leaves

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Introduction

A high yielding ability in crops is a primary objective of plant breeding and photosynthesis is the most fundamental function in relation to the dry matter production and yield in crops. To capture and utilize energy of the sun effectively and efficiently, tremendous efforts in research and practices to enlarge a leaf area per unit land and to establish an adequate canopy structure have thus far focused on the improvement of photosynthetic capacity and the dry matter production[10]. The utilization of semi-dwarf genes from the varieties Dee Geo Woo Gen and Jukkoku in rice and Norin 10 in wheat was an example of the genetic improvement in the breeding programs of high yielding varieties (HYVs) in staple crops. A number of HYVs have been accomplished in rice and wheat through the improvement and changes of canopy structure, which is occasionally called "Plant-type breeding".

In addition to the quantitative improvement as mentioned above, a qualitative improvement, which implies a genetic improvement of the photosynthetic capacity per unit leaf area, would also be one of the main targets to be achieved in crop breeding for the future. Hayashi et al.[2] reported that the low photosynthetic capacity is dominant to high capacity in rice by CO_2-gas exchange method.

This report presents a brief account on the development of multi-channel measuring method of photosynthetic capacity of a single leaf, the genetic diversity in the photosynthetic capacity of Asian rice cultivars, the inheritance of the relevant capacity detected by oxygen evolution in leaves, and the possibility for selecting rice plants with high photosynthesis.

Development of multi-channel measuring apparatus

The development of a simple, rapid and sustainable method in taking measurements of the leaf photosynthetic capacities of a large number of samples in a short period of time is required for the analysis of variations and the genetic studies of photosynthetic capacity. The CO_2-gas exchange method is suitable to detect changes of photosynthetic rate of intact leaves taking place under various environmental conditions. However, it does not suit the routine procedures in dealing with a large number of leaf samples in a limited period of time. Jones and Osmond[4] initiated a new method of an oxygen electrode for the studies on photosynthesis of intact plant leaves and reported that since carbonic acid was absorbed mainly through the cut surface of the leaf in a liquid phase, the effect of stomatal resistance on the CO_2 incorporation into the mesophyll cells was eliminated. In order to detect more precisely

the amount of oxygen evolution from leaves, a multi-channel apparatus of oxygen electrodes was earlier developed by Nagamine, et al.[7] (Plate 1 and Fig. 1). The apparatus has 6 measuring units, each of which consists of an acrylic reaction cell, a lamp with a condenser lens and a magnetic stirrer. The reaction cell with a capacity of 25 m*l* is sunk in a water bath for controlling its temperature. An amount of 24 m*l* of a 50 mM HEPES buffer solution titrated to pH 7.2 and 1 m*l* of $NaHCO_3$ as a carbon source is added to the

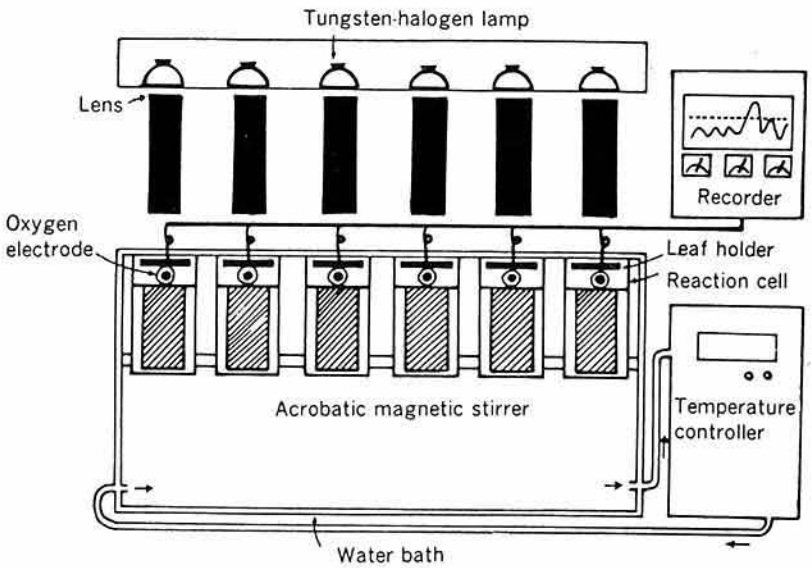

Fig. 1. Diagram of multi-channel oxygen electrodes
(Nagamine et al., 1987)

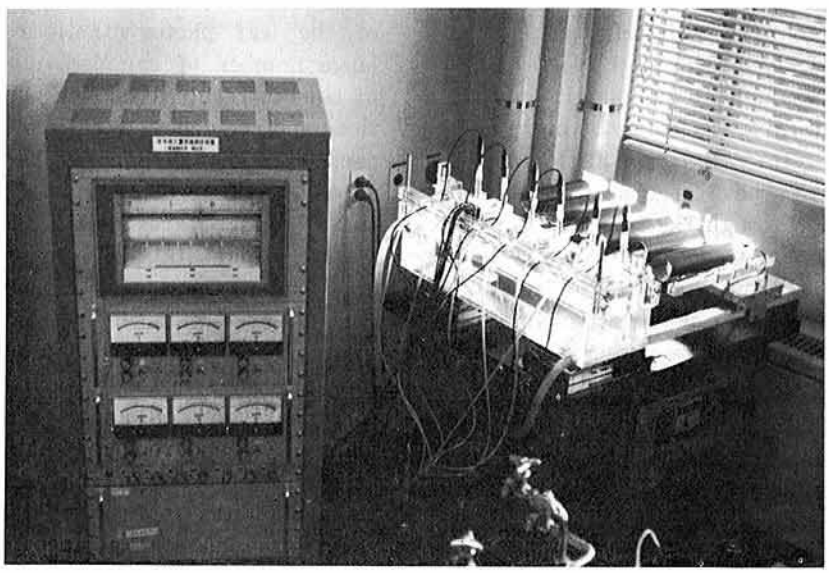

Plate 1. Multi-channel apparatus of oxygen electrodes

cell. Final concentration of $NaHCO_3$ should be 20 mM. The punched leaf disks are supported by an acrylic leaf holder and sunk in the cell. The reaction medium is continuously moved with the magnetic stirrer during the measurements. Light of a tungsten-halogen lamp (Silvania EFN 12 V–75 W) is projected to the cell through the condenser lens. Light intensity is adjusted by shifting the distance between the lamp and the cell. Temperature of the reaction medium is adjusted by using a water bath for controlling the temperature. The changes in the oxygen concentration in the reaction medium are traced by the Clark type oxygen electrodes[3]. The amount of oxygen evolution is expressed as μmol O_2/dm^2/hr.

Determination of the optimum measuring conditions

Since the values of oxygen evolution varied with the differences of the sampling time of leaf blades within a day (Table 1), a preparation method for sampling leaf materials to obtain an adequate photosynthetic capacity

Table 1. Variations of oxygen evolution with the sampling time of leaf blades

(μmol O_2/dm^2/hr)

Measuring time*	Sampling time of leaf blades			
	8 : 30$^{a.m.}$	10 : 30	12 : 30$^{p.m.}$	15 : 00
A	604±150	341± 94	239±132	181± 56
B	494±112	357±120	240± 53	172± 60

* A and B: Oxygen evolutions were measured at 30 and 60 min after sampling, respectively.

Table 2. Effects of incubation of leaf disks on oxygen evolution

(μmol O_2/dm^2/hr)

Incubation method*	Measuring time of oxygen evolution						
	9 : 00$^{a.m.}$	10 : 15	11 : 15	12 : 15$^{p.m.}$	15 : 30	16 : 25	17 : 10
A	546±46	533±120	526±98	506±28	508±48	570± 9	518±68
B	344±82	382± 41	435±34	488±60	414±38	452±54	498±38

* A and B: Leaf disks were incubated in a growth chamber and an air-controlled room for 24 hr, respectively.

Table 3. Relationship between oxygen evolution and weather conditions

Date	Mean temperature (℃)	Sunshine duration (min)	Solar radiation (cal)	Minimum humidity (%)	Precipitation (mm)	Oxygen evolution (μmol O_2/dm^2/hr)	Coefficient of variance (%)
Jul. 22	23.8	78	261	59	1.5	602	14.3
23	23.5	36	286	65	0.5	723	19.7
24	24.4	0	249	60	0.0	710	12.6
25	25.2	96	293	52	1.0	692	14.5
26	24.4	414	473	51	1.5	657	13.0
27	24.1	198	368	54	109.0	687	13.1
28	25.5	486	472	44	16.0	710	13.0
29	25.1	564	510	56	0.0	642	20.0
30	26.6	522	475	49	0.0	680	23.7
31	27.4	84	321	53	0.0	610	17.4

(Nagamine et al., 1987)

was developed. After the leaf disks were incubated in a growth chamber (illumination: 6:00 a.m.–8:00 p.m., light intensity: 40 klux, temperature: 28–22°C) for 24 hr, oxygen evolution was constant with a value of approximately 550 μmol O_2/dm^2/hr from 9:00 a.m.–5:00 p.m. (Table 2). It was also proved that such a preparation method for the leaf disks in a growth chamber provided stable results irrespective of the change in weather conditions in the field, where rice plants had been grown (Table 3).

The oxygen evolution increased with the increase of light intensity and was saturated at a light intensity of about 70 klux with a value of 480 μmol O_2/dm^2/hr. The oxygen evolution reached a maximum level at 25°C and the value slightly decreased at above as well as below 25°C. The oxygen evolution reached the maximum value of 629 μmol O_2/dm^2/hr at pH 7.2 of HEPES buffer solution and decreased at a higher level beyond pH 7.2.

From the above experiment, it is concluded that the followings are the optimum conditions for taking measurements of oxygen evolution: light intensity of 70 klux, temperature of 25°C, and HEPES buffer solution of pH 7.2. Photosynthetic oxygen evolution could be measured for 150 samples in a day by using the multi-channel apparatus under the optimum measurement conditions determined as above.

Genetic diversity of oxygen evolution in Asian rice cultivars

In regards to the varietal differences of photosynthetic capacities, 91 indigenous rice varieties which could head in Tsukuba, Japan were subjected to comparison of their oxygen evolutions. Those cultivars belong to the following four groups; Indica (25 varieties), Japonica (25), Javanica (25) and Sinica (Chinese hsien varieties) (16)[7]. The measurements of oxygen evolution were taken at the growing stage of 20–26 days after transplanting with 4 replications.

The lowest value of oxygen evolution among the 91 varieties under study was 501 μmol O_2/dm^2/hr, which took place in the variety belonging to the Javanica group, while the highest was 895 μmol O_2/dm^2/hr, which occurred in the Indica group (Table 4). The ratio of the highest/the lowest values was 1.84, indicating that there existed a large genetic diversity among the 91 rice cultivars studied. This ratio is greater than that reported by Akita (1980), who had adopted a CO_2-gas exchange method. According to Murata (1957), ±20% of deviation of photosynthetic ability existed among the 29 rice varieties at the maximum tillering stage. The above result of the present study confirms that a large genetic variation exists among the Asian rice cultivars regarding their photosynthetic capacities.

The differences of oxygen evolution among the rice varietal groups were identified (Table 4). The average value of oxygen evolution of 25 varieties in the Javanica

Table 4. Variations of oxygen evolution in the four rice varietal groups

(μmol O_2/dm^2/hr)

Varietal group	Number of varieties			
	Indica 25	Japonica 25	Javanica 25	Sinica 16
Range	598–895	651–884	501–799	664–811
Average	724	772	613	722
Variance	6,791	4,931	3,896	1,781
Highest/lowest	1.50	1.36	1.59	1.26

group was 613 μmol O_2/dm^2/hr, which was the lowest in the four groups. No significant differences were observed among the Indica, Japonica and Sinica groups. This result is comparable to the data reported by Samejima (1984), which indicated that activities of ^{13}C-discrimination of the Indica and Sinica (hsien type) varieties had been higher than the Japonica and Javanica varieties.

Inheritance of oxygen evolution

The variation of oxygen evolution was investigated on a plant basis in the segregation of the F_2 hybrid population. Two varieties, Zai Ye Qin 8 originated in China, and Tamanishiki originated in Japan, bearing high and low oxygen evolutions, respectively were crossed; the resultant F_1 and F_2 plants were subjected to analyses of oxygen evolution capacities. The values of the evolution in rice plants considerably varied

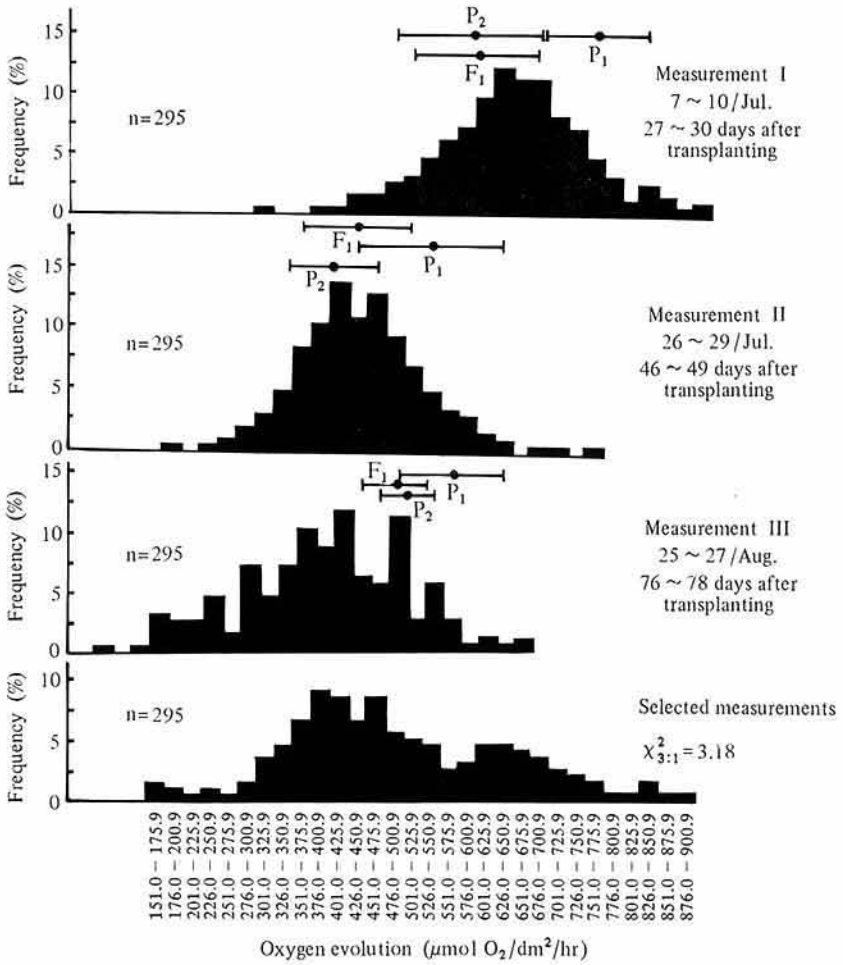

Fig 2. Distribution of oxygen evolutions in the F_2 population of Zai Ye Qin 8×Tamanishiki
Selected measurements: The data on oxygen evolutions are taken from the specific growth stage (approximately 35 days before heading) of the F_2 plants.

according to the growth stages. A great variation of growth duration in the F_2 plants was observed. In order to eliminate the effects of the fluctuations caused by the different growth stages, the oxygen evolutions were measured in the three different growth periods; 27–30 days (7–10 Jul.), 46–49 days (26–29 Jul.) and 76–78 days (25–27 Aug.) after transplanting.

Average values of oxygen evolution and standard deviations in the F_1 plants and both parents, and distribution of the values in the F_2 population at the three different growth periods are shown in Fig. 2.

The oxygen evolution of the F_1 plants was similar to that of the low capacity parent. The variation of oxygen evolutions in the F_2 population in each of the three different periods showed a normal distribution. The similar finding was reported by Ojima (1972) in the soybean hybrid. Net photosynthesis rates of the F_2 plants of Norin 1 × Harosoy and Manshu × Harosoy in soybean showed no monogenic distribution. Such a normal distribution might have been attributed possibly to the deformation caused by the differences in growth stages of each plant in the F_2 population.

In order to compare the oxygen evolutions at the same growth stage in the F_2 population, the values of oxygen evolutions around 35 days before heading dates were chosen among the three different sources for comparison of the F_2 plants (Fig. 2). The data from these specific growth stages showed clearly a binominal distribution. The segregation of oxygen evolution yielded a goodness of fit to the monogenic inheritance. This result suggests that the oxygen evolution be inherited monogenically and the low photosynthetic capacity be dominant. A high possibility of obtaining improved capacity segregants could be foreseen by selecting the F_2 plants which exhibited high values of oxygen evolution.

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