Genetic Analysis of Photosynthetic Capacity of Single Leaf Analyzed by Oxygen Polarography in Rice, *Oryza sativa* L.

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The genetic control of the photosynthetic capacity of a single leaf was studied in rice. The photosynthetic capacity was estimated as the amount of oxygen evolved that was measured by oxygen polarography using a multi-channel apparatus. The amount of oxygen evolved by the F₁ plants and the F₂ populations of the two reciprocal crosses; Zaiyeqin 8 (high capacity) × Shinshukaneko (low capacity) and Jukkoku (high) × Naxi (low), were measured. The frequency distribution of the amount of oxygen evolved which was measured at a fixed period of measurement in the F₂ populations showed a normal distribution. As the growth stages of the F₂ plants used varied widely, it is concluded that the uniformity of growth stage makes the genetic analysis difficult, and that the data obtained by the above measurements was not sufficient to analyze the genetic control. For another cross combination, Zaiyeqin 8 (high) × Tamanishiki (low), the amount of oxygen evolved by the F₁ plants, the F₂ hybrid population and the both parents were measured at three different periods of measurement. Normal distributions were obtained in the amount of oxygen evolved by the F₂ population at each measurement, too. To compare the amount of oxygen evolution at the same growth stage, the values around 35 days before heading date were chosen from the three different measurements. A bimodal distribution was found in the standardized values in the F₂ population, which suggests a single major gene controls the amount of oxygen evolution. As the standardized amount of oxygen evolved by the F₁ plants was close to that of the low capacity parent, it is concluded that the low capacity is a dominant character.

KEY WORDS: *Oryza sativa*, photosynthesis, oxygen evolution, genetic control.

Introduction

Since the main objective of plant breeding is to obtain high yielding varieties, photosynthesis is one of the most fundamental processes related to dry matter production and yield in crops. To absorb and utilize solar energy the canopy structure must be adequate and the improvement of the photosynthetic capacity in a single leaf is essential. We previously reported the existence of genetic variations in the photosynthetic capacity in indigenous Asian rice varieties by using oxygen polarography (NAGAMINE, 1991). Since a large variation of the character was observed, it may be possible to utilize a high capacity found in some varieties for breeding high yielding lines. To improve the character, it is important to analyze its genetic control. HAYASHI *et al.* (1977) applied CO₂ gas exchange method for the genetic analysis of the photosynthetic capacity in rice and pointed out that a single major gene controls the capacity and the low capacity is dominant. SAMEJIMA (1985) who studied the genetic control by measuring ¹³C-discrimination which reflects the CO₂ concentration in mesophyll (BRUGNOLI *et al.*, 1988), suggested that the low capacity of ¹³C-discrimination is a dominant character. Since there are only a few investigations on the genetic control of the photosyn-

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thetic capacity of a single leaf in rice, the present paper deals with the genetic analysis of the character. The photosynthetic capacity was estimated by the amount of oxygen evolved.

Materials and Methods

1. Genetic analysis of the amount of oxygen evolution measured at a fixed period

Two rice varieties with a high capacity of oxygen evolution and two varieties with a low capacity were selected after the determination of the amount of oxygen evolved (NAGAMINE, 1991; NAGAMINE, unpublished). The measurement conditions were temperature of 25°C and light intensity of 70 klux. Two reciprocal cross combinations; a Chinese improved variety, Zaiyeqin 8 (high capacity) × a Japanese indigenous variety, Shinshukaneko (low capacity) and a Japanese improved variety, Jukkoku (high capacity) × a Chinese indigenous variety, Naxi (low capacity) were used to investigate the genetic control for the amount of oxygen evolved. The F₁ plants, the F₂ hybrid populations and the both parents were transplanted in paddy field on June 5th in 1985 and their amount of oxygen evolved were measured at a fixed period of measurement (Table 1). At least 5 plants were measured and averaged for each F₁ plant and the both parents. Approximately 300 F₂ plants were investigated for each cross combination. Fully expanded leaf on the main shoot was sampled in the previous day of measurement and the central portion of the leaf blade was used to make leaf disks, then the disks were floated on distilled water in a glass container and incubated in a growth chamber under illumination from 6:00 a.m. to 8:00 p.m. (light intensity, 40 klux) at 28°C during daytime and 22°C at night for 24 hours (NAGAMINE *et al.* 1987). The measurement of the amount of oxygen evolution was carried out under the optimum conditions of temperature: 25°C, light intensity: 70 klux and the pH of HEPES buffer solution: 7.2 using a multi-channel apparatus (NAGAMINE *et al.* 1987).

2. Genetic analysis based on the standardized amount of oxygen evolution

To study the genetic control for the amount of oxygen evolution in detail, a cross combination between Zaiyeqin 8 (high capacity) and a Japanese indigenous variety, Tamanishiki (low capacity) was used. The F₁ plants, the F₂ population (295 plants) and the both parents were transplanted in a paddy field on June 10th in 1986 and the amount of oxygen evolution were measured at three different periods; phase I: July 7th~10th (27~30 days after

Table 1. Measurement periods of oxygen evolution and the number of F₂ plants analyzed in the four cross combinations

Cross combination	Measurement period	Number of F ₂ plants analysed
Zaiyeqin 8 (H) × Shinshukaneko (L)	July 19th-21st, 1985	297
Shinshukaneko (L) × Zaiyeqin 8 (H)	July 23rd-24th, 1985	293
Jukkoku (H) × Naxi (L)	August 5th-7th, 1985	296
Naxi (L) × Jukkoku (H)	August 1st-3rd, 1985	299

Note: H and L in the parenthesis show a high capacity and a low capacity of oxygen evolution, respectively.

transplanting), phase II: July 26th~29th (46~49 days after transplanting) and phase III: August 25th~27th (76~78 days after transplanting). The amount of oxygen evolved by 12 plants was measured for the F_1 plant and the both parents, while that of the plants of the F_2 population was measured with one replication. The heading date in each F_2 plant was recorded. The sampling and preparation methods of leaf materials and measuring conditions and the apparatus used were the same as described above.

Results

1. Genetic analysis of the amount of oxygen evolution measured at a fixed period

1) Reciprocal cross combinations of Zaiyeqin 8 $\times$ Shinshukaneko

Averaged values of oxygen evolution in Zaiyeqin 8 and Shinshukaneko were 674 and 510 $\mu\text{mol O}_2/\text{dm}^2/\text{hr}$, respectively. The amount of oxygen evolved by the F_1 plant was 537 $\mu\text{mol O}_2/\text{dm}^2/\text{hr}$, being close to the value of the low capacity parent, Shinshukaneko. The amount of oxygen evolution measured at July 19th to 21st for the F_2 plants ranged from 301 to 952 $\mu\text{mol O}_2/\text{dm}^2/\text{hr}$, extended the range of the values of the both parents. The frequency distribution of the values in the F_2 plants was similar to a normal distribution (Fig. 1).

The F_1 plants of the reciprocal cross combination, Shinshukaneko $\times$ Zaiyeqin 8, showed 479 $\mu\text{mol O}_2/\text{dm}^2/\text{hr}$, which value was also close to that of low capacity parent. The amount of oxygen evolution of the F_2 plants measured at July 23rd to 24th ranged from 226 to 973 $\mu\text{mol O}_2/\text{dm}^2/\text{hr}$, and the frequency distribution was similar to a normal distribution, too (Fig. 2).

2) Reciprocal cross combinations of Jukkoku $\times$ Naxi

The F_1 plants of the cross between Jukkoku and Naxi showed 538 $\mu\text{mol O}_2/\text{dm}^2/\text{hr}$, which was close to the averaged value of Naxi, 526 $\mu\text{mol O}_2/\text{dm}^2/\text{hr}$. The frequency distribution of the values in the F_2 plants measured at August 5th to 7th extended the range of the both

Fig. 1. Distribution of oxygen evolution in the F_2 population of the cross between Zaiyeqin 8 and Shinshukaneko.

Fig. 2. Distribution of oxygen evolution in the F_2 population of the cross between Shinshukaneko and Zaiyeqin 8.

parents and was similar to a normal distribution (Data not shown). For the reciprocal cross combination, Naxi $\times$ Jukkoku, the F_1 showed $480 \mu\text{mol O}_2/\text{dm}^2/\text{hr}$, which was close to that of Naxi. The range of the amount of oxygen evolved by the individual F_2 plants measured at August 1st to 3rd was wider than those of both parents and the frequency distribution was similar to a normal distribution (Data not shown).

The amount of oxygen evolution changes along with the growth stage (NAGAMINE, 1991) and the growth stages of the F_2 plants used varied widely. It is, therefore, concluded that the uniformity of growth stage makes the genetic analysis difficult, and that sufficient data which is able to apply the analysis could not be obtained by the above measurements.

2. Genetic analysis of the standardized amount of oxygen evolution

The frequency distributions of the amount of oxygen evolved by the F_2 plants of the cross between Zaiyeqin 8 and Tamanishiki at three different measurements are shown in Fig. 3 (a, b and c). The values of the both parents, Zaiyeqin 8 and Tamanishiki, were 761 and $592 \mu\text{mol O}_2/\text{dm}^2/\text{hr}$, respectively, at phase I. The amount of oxygen evolved by the F_2 ranged from 269 to $1,387 \mu\text{mol O}_2/\text{dm}^2/\text{hr}$, and the frequency distribution was similar to a normal distribution (Fig. 3a). The values of both parents, the F_1 plants and the F_2 population at phase II were lower than those at phase I. The amount of oxygen evolved by the F_1 plants was close to the amount recorded for Tamanishiki, and the frequency distribution of the F_2 population was similar to a normal distribution at phase II (Fig. 3b). The values at phase III in the F_2 population showed a normal but rather wide distribution (Fig. 3c). Thus a normal distribution of the amount of oxygen evolved was observed at three different periods of measurement.

As there was a range of more than 50 days in the heading date within the F_2 plants, such a normal distribution may have been associated with the deviation caused by the differences in the growth stages of the F_2 plants. The amount of oxygen evolution at the same

Fig. 3. Distribution of oxygen evolution in the F_2 population of the cross between Zaiyeqin 8 (P_1) and Tamanishiki (P_2).

growth stage in the F_2 plants should be compared. We already reported that the highest value is obtained at the growth stage of 7th or 8th leaf (NAGAMINE, 1991). Although, it is difficult to investigate the change of leaf number for each plant in the F_2 population, the value measured around 35 days before heading was adopted as standardized value for each F_2 plant.

A bimodal distribution was found in the standardized values, which suggests that a major gene controls this character (Fig. 3d). The χ^2 value for 3:1 was 3.18 which indicates that there was no significant deviation from the segregation ratio expected by a monogenic control. As the standardized amount of oxygen evolved by the F_1 plants was close to that of the low capacity parent, it is concluded that the low capacity is a dominant character.

Discussion

Since there was a large variation in the photosynthetic capacity of a single leaf estimated by the amount of oxygen evolved among rice varieties and since it may be possible to improve the character (NAGAMINE, 1991), it is necessary to analyze its genetic control. In the present experiment, the standardized amount of oxygen evolved by the F_1 plants was close to that of the low capacity parent, and no heterosis was detected for the character in the F_1 plants. HAYASHI *et al.* (1977) and SAMEJIMA (1985) reported that the low photosynthetic capacity is a dominant character and there is no heterosis for the character in rice. OJIMA (1972) and IZHAL and WALLACE (1967) reported that there is some dominance for the low capacity in soybean and common bean, respectively. On the other hand, MURAYAMA *et al.* (1982) reported that the F_1 plants of some combinations of rice varieties showed heterosis. SAKA (1985) reported that RuBPc/o activities of the F_1 plants derived from crosses between chlorina mutants and its original variety seemed to show a tendency of heterosis. HEICHEL and MUSGRAVE (1969) and MONMA and TSUNODA (1979) reported that significant heterosis was observed in the F_1 hybrids from some cross combinations in maize.

A bimodal distribution was shown in the standardized amount of oxygen evolved by the F_2 plants of the cross between Zaiyeqin 8 and Tamanishiki.

The F_2 plants segregated into 3 low capacity:1 high capacity. The segregation fitted well to a monogenic control. It was assumed that a single major gene controls the amount of oxygen evolved and that the low capacity is a dominant character.

HAYASHI *et al.* (1977) also suggested that a single major gene controls the photosynthetic capacity and that the low capacity is dominant. Their findings support our conclusion. The similar genetic control is also reported in other crop. AUGUSTINE *et al.* (1976) presented that carboxylation efficiency is controlled by a single gene conditioned by the high pigment gene in tomato. On the other hand, there are different suggestions that the photosynthetic capacity is controlled by minor genes. IZHAL and WALLACE (1967) suggested the genetic mechanism controlling the varietal difference in net CO_2 exchange rate is quantitative in common bean. OJIMA (1972) reported that the inheritance of photosynthetic capacity is quantitative in soybean.

Detailed analysis should be needed on the genetic control of photosynthetic capacity. A further definite conclusion on the inheritance of the character will be obtained by the analysis employing materials with a similar genetic backgrounds.

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酸素電極法によるイネ個葉光合成能力の遺伝分析

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著者らはこれまでに酸素電極法を用いた多点式酸素放出量測定装置を作出し, イネ葉身酸素放出量の測定方法を開発した. 91品種の在来イネの酸素放出量を測定して個葉光合成能力を調べたところ, 大きな変異が存在することを認め, アジア栽培稲の中ではジャワ型品種群が酸素放出量が低いことを明らかにした.

本報ではイネ個葉光合成能力の遺伝分析の結果を報告する. 本実験においては, 葉身から放出される酸素量を個葉光合成能力とした. 酸素放出量のスクリーニングの結果から, 高放出量親品種として中国の改良品種窄葉青8号および日本の改良品種十石, 低放出量親として日本の在来品種信州金子および中国の在来品種納西を選んだ. 窄葉青8号×信州金子および十石×納西の2組の正逆組合せのF₁植物とF₂雑種集団を作出した. これらの材料を両親とともに圃場に栽培し, 各組合せについてそれぞれ特定の一時期に酸素放出量を測定した. 測定条件は温度25℃, 照度7万ルクスとし多点式測定装置を用いて行った. いずれの組合せにおいても, F₁の酸素放出量は低放出量親の値に近かった. また, F₂雑種集団の酸素放出量は両親の値より超越した分離を示し, その分布は正規分布に似ていた. これは, 用いたF₂個体の生育ステージの変異が広く, 同じ生育ステージにおける酸素放出量がえられなかったためと考えた.

つぎに, 酸素放出量の遺伝分析を詳しく行うために, 窄葉青8号(高放出量)と日本の在来品種玉錦(低放出量)のF₁植物とF₂雑種集団を両親ともに時期をずらして3回測定した. 測定条件は前の実験と同じであった. F₁の酸素放出量は低放出量親である玉錦の値に近かった. F₂集団の酸素放出量の変異は3測定時期のいずれも両親の値の範囲より広く, その分布は正規分布に似ていた. すなわち, 同一のF₂集団の酸素放出量を測定時期をずらして3回測定しても正規分布に似た分布がえられた.

用いたF₂個体間には50日以上の出穂期の差があり, 個体の生育ステージに大きな変異があったこと, 酸素放出量は葉位によって変化すること(前報)の理由から, F₂個体の生育ステージを揃えて酸素放出量を比較する必要があった. F₂全個体の葉齢を経時的に追跡することは困難であったので, F₂個体の出穂期に基づいてそれらの酸素放出量を標準化した. すなわち, F₂個体について3回の測定でえられた酸素放出量の中から, 出穂前35日に近い測定時期の値を標準化した放出量とした. その結果, 標準化した酸素放出量は二頂分布とみなしてもよい分布を示した. χ^2 検定の結果, 単一遺伝子のF₂世代における期待分離比に適合したので, 酸素放出量は一対の主働遺伝子によって支配されると考えた. また, 標準化したF₁個体の値は低放出親の値に近かったことから低放出量は優性であると結論した.