

Photochemical Reactions of Rice Chlorina Mutant, CMV-44*

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Abstract : The light saturations of photochemical reactions of rice chlorina mutant, CMV-44, which lacks chlorophyll b entirely, were examined as well as those of the barley chlorina *f2* mutant. The efficiencies of the photoreactions of photosystem I and photosystem II were lower in a rice chlorina mutant than in a wild-type plant. Based on the findings in the present investigation, the plant leaves with higher chlorophyll b content, having a larger photosynthetic unit, are considered to exhibit relatively high photosynthetic activity in low light conditions as in the plant canopy, under the cover of protected cultivation and in the winter season.

Key words : Chlorina mutant, CMV-44, Photochemical reaction, Rice.

水稻のクロリナミュータント, CMV-44 の光化学反応: 狩野広美・小泉美香・桂 直樹*・稲田勝美** (農業生物資源研究所・* 現野菜茶業試験場・** 現鳥取大学砂丘利用研究施設)

要 旨: クロロフィル b を完全に欠く水稻のクロリナミュータント, CMV-44 の光化学反応の光飽和曲線を, オオムギのクロリナミュータントである Chlorina *f2* とともに調べた。

SDS-ポリアクリルアミドゲル電気泳動によってクロロプラストのチラコイド膜上の蛋白質を比較すると, 通常型品種に存在する集光性クロロフィル a/b 蛋白複合体と光化学系 I のアンテナクロロフィル蛋白複合体がクロリナミュータントでは欠けていた。このためにクロリナミュータントのクロロフィル a/b 比は高く, 光合成単位のサイズ (クロロフィル/P700 比) は小さくなっていた。

水稻の単離クロロプラストを用いて, NADP 還元による光化学系 I の活性と, フェリシアン化カリの還元による光化学系 II の活性を測定して光飽和曲線を求めると, 通常型品種である農林 8 号の方が低照度領域での活性が高く, CMV-44 より低照度で最大活性に達した。

次に, 閃光分光分析器により生葉の P700 の吸光度変化を測定した。測定光によって酸化されている P700 の割合 (H/P) は系 I の反応効率を示すが, これを数種的水稻の栽培品種とオオムギについて測定すると, クロロフィル/P700 比が大きいものほど系 I の反応効率は高かった。さらにこの吸光度変化から, 系 I の光飽和曲線をシミュレートすると, 低照度領域におけるクロリナミュータントの反応効率は通常型品種より低く, 単離クロロプラストで得られた結果が確認された。一方, 最大活性は, 水稻ではほぼ同じであったが, オオムギの場合, クロリナミュータントは通常型品種の約 1/2 になっていた。

本研究の結果より, クロロフィル b 含量が高く光合成単位の大きな作物葉は, 葉の混み合った群落中, 施設栽培および冬期のような低照度条件下においても高い光合成活性を示すであろうと考えられる。

キーワード: クロリナミュータント, 光化学反応, CMV-44, 水稻。

Plant mutants lacking a certain photosynthetic pigment are considered to be useful materials to study photochemical reactions of crop leaves. Chlorophyll b deficient mutants of rice plant were reported by Inoue et al.⁷⁾,

Inada and Katsura⁸⁾ and Terao et al.¹⁵⁾, some of which totally lack chlorophyll b and others contain reduced amount of this compound. Chlorophyll b, light-harvesting pigment, locates in the light-harvesting chlorophyll a/b protein complexes on the thylakoid membranes of chloroplasts. The trace of SDS-polyacrylamide gel electrophoresis demonstrated that the apoproteins of light-harvesting chlorophyll a/b protein complexes decreased in the thylakoid membranes of rice chlorina mutants compared to that of wild-type plants, and in some cases, it was found in a negligible amount¹⁶⁾. The deficiency of light

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-harvesting chlorophyll and associating apo-proteins results in smaller amount of antenna chlorophyll to a photoreaction center, i.e. smaller photosynthetic unit size in chlorina mutants than in wild-type plants. This is known to decrease the efficiency of photoreactions to light energy¹⁴⁾ as reported in barley chlorina *f2* mutant²⁾, pea chlorophyll deficient mutant⁵⁾ and tobacco aurea mutant¹³⁾. However, the photochemical reactions of rice chlorina mutants have not been investigated extensively.

In the present investigation, the light saturations of photosystem I and photosystem II activities were examined using a rice chlorina mutant, CMV-44, which was reported to be entirely lacking chlorophyll *b* by Inada and Katsura⁸⁾, with relation to the photosynthetic unit size and apoproteins of light-harvesting chlorophyll *a/b* protein complexes, as well as those of the barley chlorina *f2* mutant. The efficiencies of photoreactions were lower in the rice chlorina mutant, CMV-44 than in a wild-type plant with a larger photosynthetic unit size.

Materials and Methods

Plants ; Rice plants (*Oryza sativa* L.), cv. Norin 8 and a chlorina mutant strain, CMV-44 were grown in a growth chamber with natural light at 28°C in the day time and 23°C at night. Barley plants (*Hordeum vulgare*), wild-type and chlorina *f2* mutant strain were grown in a growth chamber with natural light at 25°C in the day time and 20°C at night.

Preparation of chloroplasts ; Leaves of 3-week-old plants were homogenized for 10 s in a blender with a medium containing 0.4 M sucrose, 50 mM Tricine-NaOH (pH 7.8) and 50 mM KCl. The homogenate was filtered through four layers of cheesecloth and centrifuged at $600\times g$ for 2 min to remove debris. The chloroplasts were then collected by centrifugation at $5,000\times g$ for 3 min.

Activities of photosystem I and photosystem II in isolated chloroplasts ; The rates of photochemical reactions were measured by a double beam spectrophotometer (Shimadzu UV 3000) with equipment for illumination from a halogen lamp. Relative intensity of actinic light (white light) was decreased by neutral density filters. The reduction of NADP upon projection of actinic light to the chloroplast suspension in 30

mM Tris-buffer (pH 8.3) containing 40 mM NaCl, 170 μ M NADP, 5 μ M ferredoxine, 70 μ M DCIP, 3 mM ascorbate, 10 μ M DCMU and 0.4 M sucrose was traced by increasing A_{340} for the measurement of photosystem I activity. The Hill reaction of the chloroplast suspension in 30 mM Tris-buffer (pH 8.0) containing 7 mM NaCl, 1.7 mM ferricyanide and 0.4 M sucrose was traced by decreasing A_{420} for the measurement of photosystem II activity. Chloroplasts containing chlorophyll from 10 to 15 μ g/ml were used for each measurement.

SDS-polyacrylamide gel electrophoresis of polypeptides in thylakoid membrane ; Polypeptides of chloroplast membrane were analysed as described by Laemmli¹¹⁾. Samples were incubated in a medium containing 8 M urea, 2.5% SDS and 5% β -mercaptoethanol and 125 mM Tris-HCl (pH 6.8) at room temperature for 1 h, then run in 12.5% acrylamide gels for 4 h.

Measurements of flash-induced absorption change in intact leaves ; Measurement of the flash-induced absorption changes of P700 in intact leaves (1 \times 2 cm segment) was carried out by a flash spectrophotometer (Union Giken Kogyo Co. Ltd.) according to the method described in a previous paper¹⁰⁾ at 25°C. P700 content in a leaf was determined from the amplitude of the signal assuming that the extinction coefficient of P700 was 64 per mM⁶⁾. Chlorophyll contents were measured according to the method of Arnon¹⁾.

Results and Discussion

The content of chlorophyll and P700 per unit leaf area (cm²), photosynthetic unit size (chlorophyll/P700) and chlorophyll *a/b* ratio in the leaves of rice and barley wild-type plants and chlorina mutants are listed in Table 1. CMV-44, rice chlorina mutant showed a chlorophyll *a/b* ratio higher than 45, while Norin 8, a wild-type plant had a ratio of 3.95, which were comparable to the values reported by Inada and Katsura⁸⁾. A similar tendency was found in barley plants ; the chlorophyll *a/b* ratio of chlorina *f2* mutant was more than 100 while that of wild-type barley 3.49. The content of P700, which indicates the amount of photosynthetic unit, did not differ between rice wild-type plant and chlorina mutant. On the other hand, P700 content in chlorina *f2* mutant was approxi-

Table 1. P700 content, chlorophyll/P700 ratio and chlorophyll a/b ratio in the wild-type and chlorina mutant plants of rice and barley.

Plant	Chlorophyll content nmol/cm ²	P700 content pmol/cm ²	Chlorophyll/P700	Chlorophyll a/b
Rice				
Norin 8	48.7	96.1	506.6	3.95
CMV-44	25.6	90.2	283.7	46.24
Barley				
Wild-type	71.0	140.7	504.6	3.49
Chlorina <i>f</i> 2	27.4	75.5	362.9	> 100.0

mately half of that in wild-type plant of barley. The sizes of photosynthetic unit in chlorina mutants were smaller than those of wild-type plants as reported in the case of rice by Inoue et al.⁷⁾ and of barley by Thornber and Highkin¹⁷⁾. From the quantitative relation between the decrease of chlorophyll contents and the increase of chlorophyll a/b ratios, it could be estimated that the content of chlorophyll b and that of a part of chlorophyll a were appreciably reduced in the leaves of chlorina mutants.

Fig. 1 shows the SDS-polyacrylamide gel electrophoresis of polypeptides in the rice and barley chloroplast membranes. Rice chlorina mutants lacked the 24 and 25 kDa polypeptides related to light-harvesting chlorophyll a/b protein complex and 22 kDa polypeptide which is constituents of the photosystem I peripheral antenna complex in the wild-type chloroplast membranes as reported by Terao et al.¹⁶⁾. In the case of barley, polypeptides related to light-harvesting chlorophyll a/b protein complex of 25 and 27 kDa and those of the photosystem I peripheral antenna complex of 21 and 22 kDa^{3,4,12)} were not found in chlorina *f*2 mutant. Therefore, the decrease in the photosynthetic unit size in rice and barley chlorina mutants (Table 1) was ascribed to the decrease in the light-harvesting chlorophyll a/b protein complex and photosystem I peripheral antenna complex associated with the lack of chlorophyll b (Fig. 1).

The dependency of photochemical reactions of isolated chloroplasts on the light intensity was examined. The light saturation curves of NADP and ferricyanide reduction which indicate the activities of photosystem I and

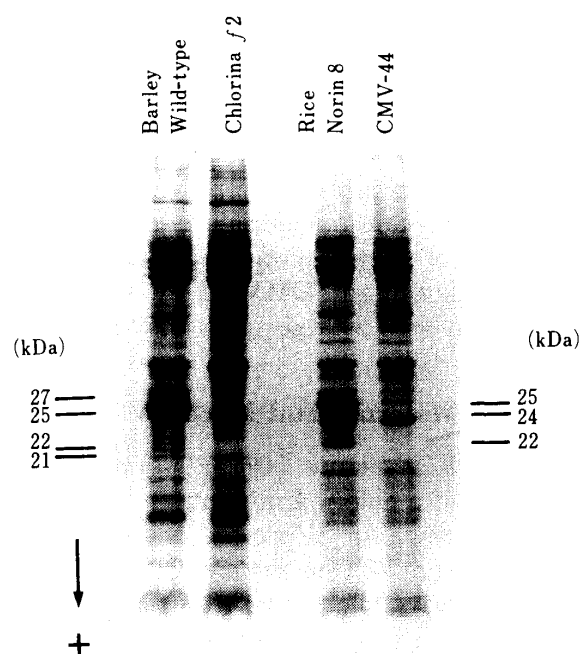


Fig. 1. SDS-polyacrylamide gel electrophoresis of chloroplast membranes in the wild-type plants and chlorina mutants of rice and barley.

photosystem II, respectively, are shown in Figs. 2 and 3. Norin 8, wild-type rice showed higher activities at the low light intensity region and reached the maximum value at a lower light intensity than CMV-44, chlorina mutant. The efficiency of light capture in chlorina mutant was considered to be low because of the deficiency of chlorophyll b as reported in barley chlorina *f*2 mutant²⁾ and pea chlorophyll deficient mutant⁵⁾.

The absorption changes associated with the oxidation and the reduction of P700 measured in the leaves of rice and barley wild-type plants and chlorina mutants by a flash

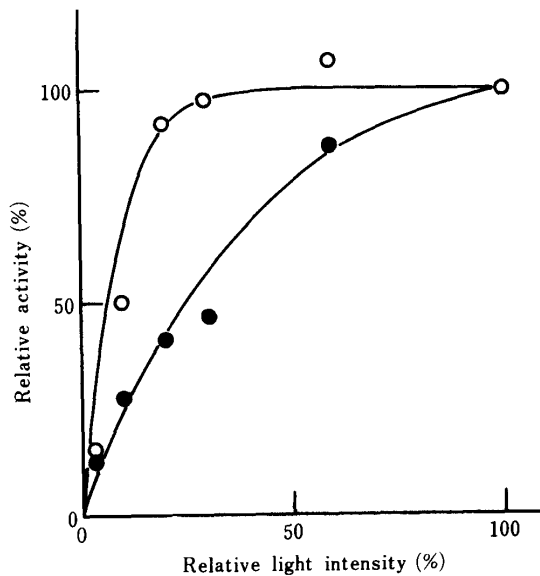


Fig. 2. Light saturation curves of photosystem I activity in the isolated chloroplasts from wild-type rice plant (—○—) and chlorina mutant (—●—).

Activities were measured by the increase of A_{340} . The intensity of the light at a level of 100% was $1.0 \times 10^2 \text{ W/m}^2$.

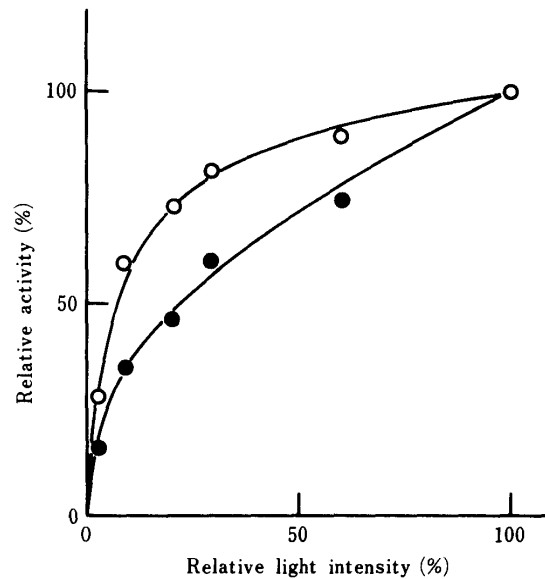


Fig. 3. Light saturation curves of photosystem II activity in the isolated chloroplasts from wild-type rice plant (—○—) and chlorina mutant (—●—).

Activities were measured by the decrease of A_{420} . The intensity of the light at a level of 100% was $4.5 \times 10^2 \text{ W/m}^2$.

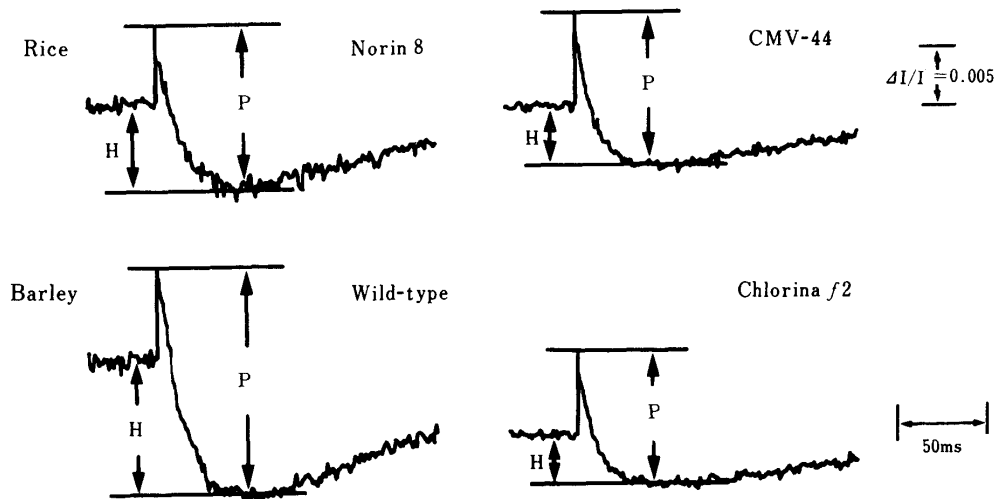


Fig. 4. Flash-induced absorption changes in the leaves of rice and barley wild-type plants and chlorina mutants.

P indicates the total amount of P700 and H the amount of P700 oxidized by detecting light.

spectrophotometer¹⁰⁾ using actinic flash light at a saturation intensity are shown in Fig. 4. The upward signal indicates the oxidation of P700 and the downward one the reduction of it. The amplitude of the signal (P) indicates the total amount of P700. The ratio of oxidized P700 by the detecting light to the total amount (H/P) in the wild-type rice, Norin 8

was remarkably higher than that in chlorina mutant, CMV-44. A similar tendency was found in the leaves of barley wild-type plant and chlorina *f2* mutant.

The oxidation of P700 by the detecting light is considered to be due to the high rates of P700 oxidation by the detecting light and slower electron inflow into P700 from the

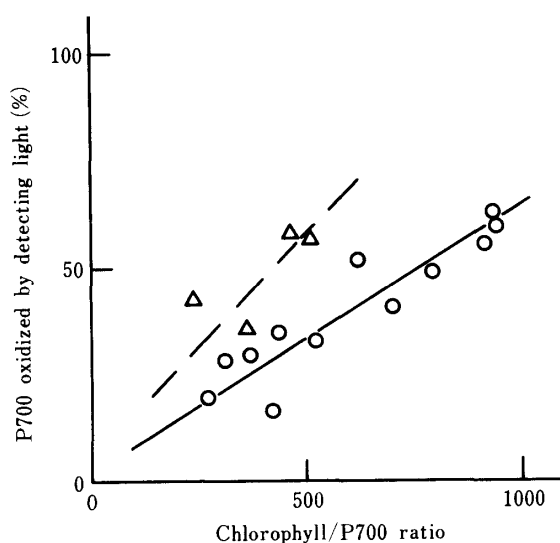


Fig. 5. Relationship between photosynthetic unit size and the percentage of oxidized P700 in the leaves of rice (—○—) and barley (---△---) plants.

photosystem II through the rate limiting step of plastoquinone. The half-life times of the P700 dark reduction, i.e. the rate of electron transport, were almost the same (approximately 8 ms) in both wild-type and chlorina mutant plants. Therefore, the discrepancy in the oxidation ratio of P700 by the detecting light was ascribed to the difference in the efficiency of light capture by the photosystem I. Fig. 4 shows that the efficiency of light capture was higher in wild-type plants than in chlorina mutants.

The efficiency of photoreaction by photosystem I was examined using several rice cultivars including chlorina mutants and also barley wild-type plant and chlorina *f2* mutant. The percentages of oxidized P700 by the detecting light was almost proportional to the chlorophyll/P700 ratio, photosynthetic unit size (Fig. 5), indicating that the efficiency of photoreaction rised along with the increase in the amount of antenna chlorophylls.

The relationship between the ratio of P700 oxidation (H/P in Fig. 4) and the intensity of detecting light was reported to be expressed by the formula $(1 - e^{-kl})$, where k is the rate constant and I is the intensity of detecting light⁹. Accordingly, the amount of P700 oxidized (H in Fig. 4) is given as follow,

$$H = P \times (1 - e^{-kl})$$

where maximum rate (P) corresponds to the total amount of P700 (P in Fig. 4) in the leaf.

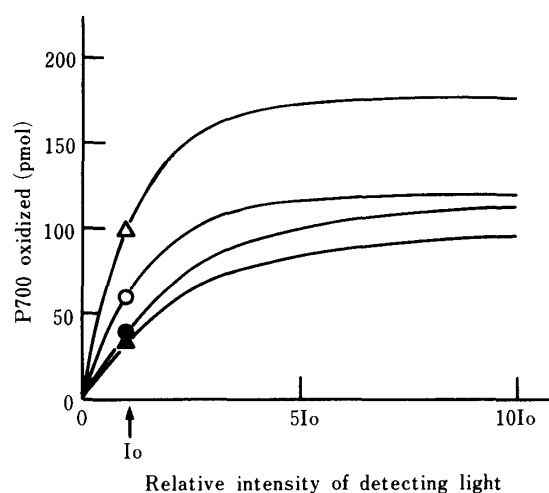


Fig. 6. Simulated light saturation curves of P700 oxidation.

Oxidation ratio of P700 in the leaves of rice wild-type plant (—○—) and chlorina mutant (—●—) and barley wild type plant (—△—) and chlorina mutant (—▲—) were measured using the detecting light of 0.2 W/m².

By using the above relation, the light saturation curve of P700 oxidation by detecting light could be simulated from the measurement at a certain light intensity (I_0) as shown in Fig. 6.

The simulated curve was higher in the wild-type rice, Norin 8, than in the chlorina mutant, CMV-44, in the low light region in spite of the fact that there was no significant difference in maximum rate. In the case of barley, besides the ratio of P700 oxidation being higher in the wild-type plant than in the chlorina mutant at low light intensities, the maximum rate of chlorina mutant was approximately a half of that of wild-type plant. The finding in isolated chloroplasts (Fig. 2 and Fig. 3) that the existence of light-harvesting pigment, chlorophyll b is necessary to collect efficiently the radiation energy, was confirmed by simulated light saturation curves from the results of the experiments by flash photolysis in intact leaves.

The oxidation rate of P700 is considered to correspond with the ability to supply reducing power (NADPH₂) and high energy compound (ATP) required for the fixation of CO₂ in the progression of photosynthesis which are generated by the mechanisms coupled with the photosynthetic electron transport *in vivo*. Consequently, the light saturation curves of P700 oxidation enable us to compare potential

activities of photosynthesis among plant leaves though they do not indicate the activity of CO₂ fixation directly. Taking into account the fact that plant leaves with the lower chlorophyll a/b ratio showed the larger photosynthetic unit size¹⁰⁾ and the findings in the present investigation, the plant leaves with higher chlorophyll b content are thought to exhibit relatively high photosynthetic activity under low light conditions as in the plant canopy, under the cover of protected cultivation, and in the winter season.

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