Photosynthetic characteristics of a new yellow-green mutant with high photosynthetic rate in rice (*Oryza sativa* L.)

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Abstract

Contents of chlorophylls and carotenoids in yellow-green mutant Biao 810S were approximately half those in control type 810S. Biao 810S had fewer grana lamellae and looser structure than 810S, lower volume of starch granules in chloroplasts, but under high temperature and high irradiance the net photosynthetic rate (P_N) of Biao 810S was higher than that of 810S. The chlorophyll fluorescence parameter F_v/F_m of Biao 810S was little higher and photochemical quenching q_P was obviously higher than those of 810S. No significant differences in P_N and biomass were observed in their hybrid combination. The yellow-green mutant phenotype may be a useful genetic marker of P(T)GMS rice used for hybrid seed production.

Additional key words: biomass; chlorophyll fluorescence; chloroplast ultrastructure; photochemical quenching; respiration rate; starch granules.

Introduction

In plants, chloroplast mutations occur frequently (Melis and Thielen 1980, Bellemare *et al*. 1982, Terao *et al*. 1985, Ghirardi and Melis 1988, Greene *et al*. 1988, Jarvis and Chen 1998, Zhou *et al*. 2006), and some of these are artificially induced. Jung *et al*. (2003) detected some photo-induced chloroplast mutants by using T-DNA injections. In these mutants, the chloroplasts were irregularly organized and the thylakoid membranes were severely disrupted; however, there was no change in the number of chloroplasts. Almost all chloroplast mutants exhibit low photosynthetic efficiency and competition ability, particularly etiolation and albino mutants which cannot grow normally (Dai *et al*. 2000, Tian *et al*. 2002, Guo *et al*. 2003, Chen and Chu 2006). Therefore, these mutants are rarely utilized for hybrid seed production.

Biao 810S is a natural yellow-green mutant selected from the thermo-sensitive genic male-sterile (TGMS) rice line 810S by Huaihua Vocational and Technical College of Hunan. Its typical characteristic is permanent yellow-green colour. Apart from leaf colour, no phenotypic difference is recorded comparing to the wild type 810S. Determining the proportion of self-crossed plants in TGMS lines is very important for testing the genetic purity of two-line hybrid rice seeds; such testing is carried out during whole growth season and is time-consuming and expensive. The yellow-green leaf can be identified at seedling stage. Therefore, the seed purity tests can be completed within a week by using the yellow-green leaf as a marker. This marker is therefore of great value in hybrid rice. We studied the photosynthetic characteristics and biomass of this mutant.

Materials and methods

Plants: Biao 810S and its hybrid combinations Biao 810S/D100 and Biao 810S/R96-1 were supplied by the Huaihua Vocational and Technical College of Hunan. We considered the basic 810S and the hybrid combinations as controls. Each material was transplanted in 3 plots, at 500 plants per plot, and the plants were managed with the normal amounts of fertilizer and water.

Pigments and chloroplast ultrastructure: Chlorophyll (Chl) content and Chl *a/b* ratio were measured using the method of Arnon (1949). For chloroplast ultrastructure determination, leaves were sampled from individual plants of accordant growth and immediately pre-fixed in 2.5 % glutaraldehyde buffered with 0.1 M phosphate buffer (pH = 7.2) and post-fixed in 1 % osmium tetroxide

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buffered with the same phosphate buffer. All specimens were dehydrated through a graded series of alcohol and embedded in Spurr's resin. Ultrathin sections were cut using a *Leica* ultramicrotome, double stained with uranyl acetate and lead citrate, and subsequently observed using a *JEM-1230* transmission microscope and photographed.

Gas exchange of 10–12 fully expanded flag leaves was determined during 09:00–11:00 (Beijing time) in mid-August (heading stage). The net photosynthetic rate (P_N) was measured at the irradiance of 1 500 μmol m^{-2} s^{-1}, temperature of 30 °C, and the natural CO_2 concentration. For making the irradiance response curve, P_N was measured at photosynthetic photon flux densities (PPFD) of 2 000, 1 800, 1 600, 1 400, 1 200, 1 000, 800, 600, 400, 200, 100, 50, 25, and 0 μmol m^{-2} s^{-1} from a *Li-Cor* LED irradiation source, and CO_2 concentration was kept at 400 μmol mol^{-1} with a *Li-Cor* CO_2 injection system; leaf temperature was 30 ºC. For making the curve of photon-saturated P_N to intercellular CO_2 concentration (C_i), P_N values were measured at CO_2 concentrations of 400, 300, 200, 100, 50, 400, 400, 500, 600, 700, and 800 μmol mol^{-1} in turn, and PPFD was kept at 1 500 μmol m^{-2} s^{-1} from a *Li-Cor* LED irradiation source; leaf temperature was 30 ºC. The dark respiration rate (R_D) was measured under natural CO_2 concentration at 21:00 h. At the tillering stage, R_D was measured under different temperatures and different stages at the temperature of 30 °C. The above mentioned measurements were done for 3 d and 5 times a day.

Chl fluorescence kinetics: The minimal initial fluorescence (F_0) and maximal fluorescence (F_m) were measured with *Li-6400* fluorometer after dark adaptation for 20 min. Then minimal fluorescence (F_0') and maximal fluorescence under irradiation (F_m') were measured after adaptation to irradiance for 1 h. The following fluorescence parameters were calculated: $q_P = (F_m' – F_s)/(F_m' – F_0')$; $q_N = (F_m – F_m')/(F_m – F_0)$.

Measurement of major agricultural traits: Ten plants were randomly sampled to measure the number of tillers, and plant height and biomass (dried at 80 °C).

Statistical analysis: For parametric data, an analysis of variance (ANOVA) was used by *Excel*. The *t*-test significance was set at $\alpha = 0.05$ and 0.01 for all tests.

Results

Photosynthetic pigments: Chl *a*, *b*, and carotenoid contents of Biao 810S were lower than those of 810S, and the total photosynthetic pigment content was approximately 50 % of the control. No significant differences were observed in the ratios of Chl *a/b* and Chls/carotenoids (Table 1).

Chloroplast ultrastucture was observed at different stages. The number of grana lamella in Biao 810S was lower and their structure relatively looser when compared with 810S. The volume of starch granules in the chloroplasts also diminished (Fig. 1).

***P*$_N$** of Biao 810S was 27.50 μmol(CO_2) m^{-2} s^{-1}, which was 24.49 % higher than that of 810S (Fig. 2). However, no significant difference was observed between hybrid combinations derived from same female parent.

Table 1. Comparison of photosynthetic pigment contents [g kg^{-1}(FM)] in Biao 810S and 810S. Chl – chlorophyll. **,**t*-test significance at 0.01 and 0.05 levels, respectively.

	Chl *a*	Chl *b*	Carotenoids	Chl *a/b*	Carotenoids [%]
810S	2.46 ± 0.17	1.01 ± 0.07	0.83 ± 0.11	2.44 ± 0.24	18.6
Biao 810S	1.27 ± 0.09**	0.53 ± 0.05**	0.43 ± 0.08**	2.39 ± 0.14	19.4

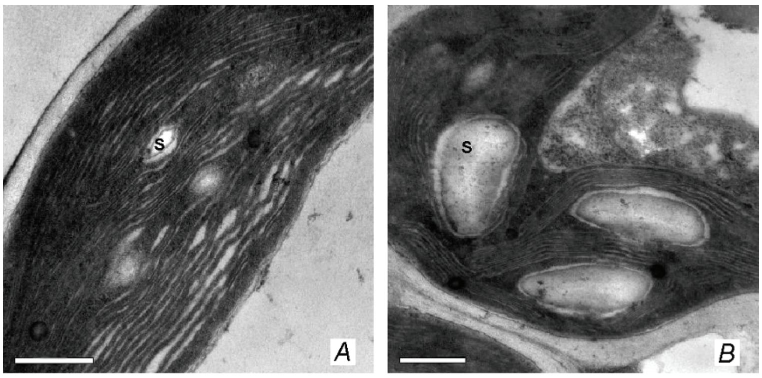

Fig. 1. Chloroplast ultrastructure of (*A*) Biao 810S at booting stage and (*B*) 810S. *A*: Less grana lamellae and looser structure, with more and smaller starch grains (S). *B*: More grana lamellae and tighter structure with fewer and bigger starch grains. Scale bars: 0.5 μm.

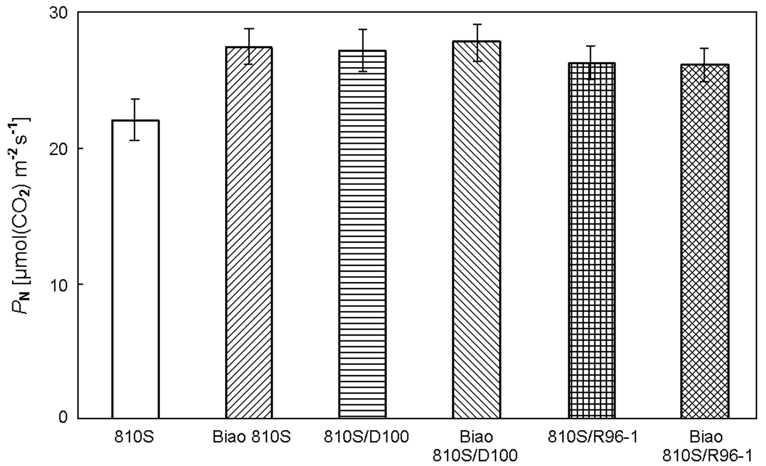

Fig. 2. Comparison of net photosynthetic rate (P_N) at 1 500 μmol m^{-2} s^{-1}, temperature of 30 °C, and natural CO_2 concentration of Biao 810S, 810S, and hybrid combinations.

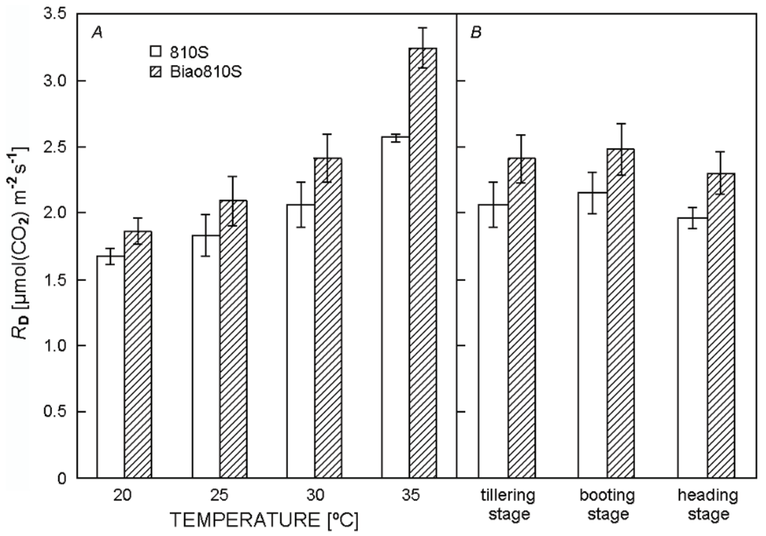

Fig. 3. Comparison of dark respiration rate (R_D) between Biao 810S and 810S at (*A*) different temperatures at tillering stage and (*B*) at different stages measured at 30 °C.

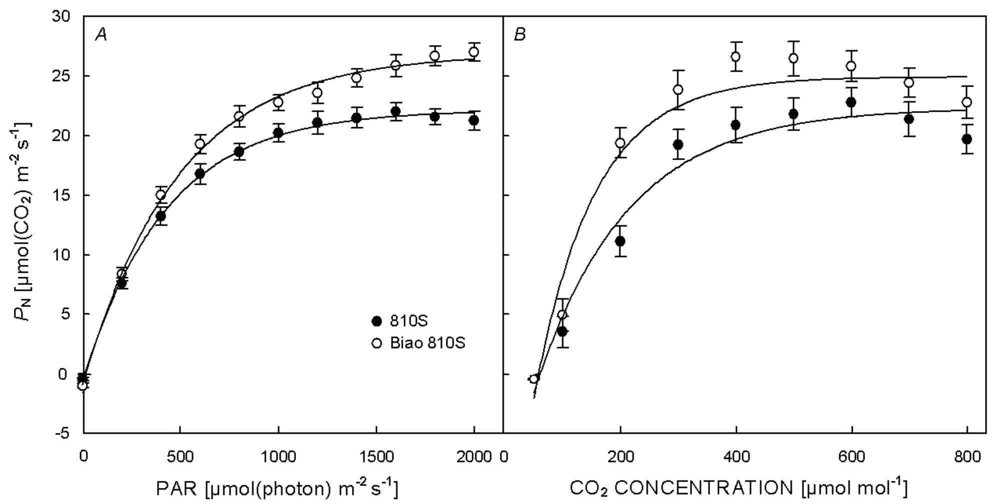

Fig. 4. Net photosynthetic rate (P_N) of 810S and Biao 810S under (*A*) different irradiances or (*B*) different CO_2 concentrations.

R_D at the tillering stage of both Biao 810S and 810S increased with temperature. However, R_D of Biao 810S was higher than that of 810S at four different temperatures (Fig. 3*A*). At 30 °C, R_D of Biao 810S was higher than that of 810S at the tillering, booting, and heading stages (Fig. 3*B*).

Table 2. Comparison of chlorophyll fluorescence kinetics between Biao 810S and 810S. **,*t-test significance at 0.01and 0.05 levels, respectively.

	F_v/F_m	F_v'/F_m'	q_P	q_N	Φ_{PS2}
Biao 810S	0.788 ± 0.003	0.627 ± 0.010**	0.743 ± 0.010**	0.462 ± 0.010**	0.466 ± 0.006**
810S	0.773 ± 0.009	0.491 ± 0.010	0.680 ± 0.010	0.712 ± 0.007	0.343 ± 0.007
±%	1.94	27.70	9.26	–35.11	35.86

Table 3. Comparison of major agronomic characters between Biao 810S, 810S, and hybrid combinations at different stages.

	Tillering Height [cm]	Biomass [g]	Booting Height [cm]	Biomass [g]	Maturing Height [cm]	Biomass [g]
810S	55.7 ± 6.11	5.32 ± 0.56	77.8 ± 6.68	21.1 ± 3.33	91.0 ±6.38	47.5 ± 3.46
Biao 810S	55.5 ±5.58	5.23 ± 0.88	77.4 ± 7.12	20.8 ± 2.25	84.7 ± 7.39	46.1 ± 4.26
810S/R96-1	71.6 ± 6.65	9.64 ± 1.23	96.9 ± 5.09	23.9 ± 2.36	100.0 ± 6.39	51.5 ± 3.33
Biao 810S/R96-1	70.3 ± 5.32	8.61 ± 1.45	95.1 ± 5.36	23.6 ± 2.58	99.3 ± 6.47	49.7 ± 3.42
810S/D100	68.5 ± 5.47	8.82 ± 1.11	97.1 ± 5.28	24.3 ± 2.12	103.4 ± 5.28	51.2 ± 3.27
Biao 810S/D100	66.1 ± 6.07	9.42 ± 1.78	96.4 ± 5.43	25.5 ± 2.48	99.7 ± 5.67	52.1 ± 3.29

Irradiance- and CO_2-response curves: P_N of 810S increased with irradiance (Fig. 4*A*). When irradiance reached 1 600 μmol m^{-2} s^{-1}, P_N peaked, and then decreased even if irradiance continued to increase. No significant photon saturation phenomenon was recorded for Biao 810S even at 2 000 μmol m^{-2} s^{-1}. Under temperature of 30 °C and irradiance of 1 500 μmol m^{-2} s^{-1}, P_N of all plant types increased as the CO_2 concentration increased until 400 cm^3 m^{-3}, then P_N declined with further increase in CO_2 concentration (Fig. 4*B*). The CO_2 compensation concentration of Biao 810S was higher than that of 810S; however, P_N was higher than that of 810S at the same CO_2 concentration.

Chl fluorescence: F_0, F_m, F_0', and q_N of Biao 810S were all lower than those of 810S, but F_m', F_v'/F_m', and q_P were obviously higher than those of 810S (Table 2). Thus Biao 810S had higher efficiency of transforming chemical energy after capturing photon energy.

Agronomic traits were studied at all growth stages: plant height and biomass of Biao 810S were slightly lower than those of 810S. No significant differences were observed in the tillering ability, plant type, and growth duration among Biao 810S, 810S, and hybrids derived from Biao 810S and 810S (Table 3).

Discussion

Although the photosynthetic pigment contents in the yellow-green mutant Biao 810S were approximately half those of 810S, it possessed integral chloroplast structure for undertaking photosynthesis and hence grew normally. Decrease in the number of grana lamella might cause insensitivity to inhibition by high irradiance. In the Chl fluorescence parameters, F_v/F_m reflected photosystem 2 (PS2) latent maximum quantum capture efficiency, F_v'/F_m' showing PS2 actual quantum efficiency, and q_P PS2-chemical energy transmitting efficiency after capturing photon energy (Roháček and Barták 1999). Biao 810S had similar F_v/F_m with 810S, but a higher F_v'/F_m' and q_P, which indicated higher photochemical efficiency of Biao 810S. Thus, Biao 810S shows a higher photosynthesis ability at high temperature and can be utilized in breeding for high photosynthesis.

The yellow-green phenotype of Biao 810S is typical by a decrease in the photosynthetic pigment contents. The combinations of 810S with D100 or R96-1 are widely used early hybrid rice combinations. P_N and major agronomic traits of hybrid combinations derived from Biao 810S and 810S with the same male parent were almost identical. Analysis of the photosynthetic pigment contents and P_N demonstrated no direct relationship between them. High irradiance and high CO_2 concentration inhibited photosynthesis to a lesser extent in Biao 810S than in 810S. Although the basal metabolism of Biao 810S was higher than that of the control 810S, Biao 810S utilized solar energy more efficiently and its P_N was higher than that of 810S. Hence we conclude that Biao 810S is an elite genetic marker TGMS rice line in hybrid seed production.

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