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Comparison of some photosynthetic characters between two hybrid rice combinations differing in yield potential

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Abstract

Photosynthetic characteristics of two hybrid rice combinations, Peiai 64S/E32 and Shanyou 63, were compared at the panicle differentiation stage. As compared with Shanyou 63, the new combination Peiai 64S/E32 showed a significantly higher net photosynthetic rate (P_N), apparent quantum yield of carbon assimilation (Φ_c), carboxylation efficiency (CE), and photorespiratory rate (R_P) as well as leaf chlorophyll content, but a significantly lower dark respiration rate (R_D) and compensation irradiance (I_c). It also showed a slightly higher photochemical efficiency (F_v/F_m and $\Delta F/F_m$') of photosystem 2, a lower non-photochemical quenching (q_N), and a similar CO_2 compensation concentration (Γ) as compared to Shanyou 63.

Additional key words: carboxylation efficiency; chlorophyll; compensation CO_2 concentration; compensation irradiance; dark respiration; net photosynthetic rate; *Oryza sativa*; photosystem 2; quantum yield; stomatal conductance.

Introduction

Photosynthesis is the most important basis of the formation of crop yield. There are two possible ways to enhance yield potential: increasing canopy photosynthesis and increasing leaf photosynthesis. Further improvement in canopy photosynthesis by breeding of fine plant type, however, is difficult because most high-yielding cultivars are close to the optimum canopy architecture. Therefore, increasing single-leaf photosynthesis could be the only way to substantially enhance yield potential (Peng 2000). Although the enhancing of photosynthetic rate is possible by using transgenic plants, there are still many questions to be resolved (Paul *et al.* 2001). The proper choice of targets of crop engineering depends on a better understanding of the regulatory mechanisms of photosynthesis. One of the important strategies to understand these mechanisms may be the quest of the causes of differences in leaf photosynthetic rate among interspecies and/or intraspecies of crops.

The variability of kinetic parameters of ribulose-1,5-bisphosphate carboxylase (RuBPC) and the ratio of its protein to total soluble protein varies little among 25 cultivars of rice (Makino *et al.* 1987). Among the 32 cultivars bred during 1882-1976 in Japan, a significant positive correlation between flag leaf photosynthesis two weeks after heading and the released year of cultivar was found (Sasaki and Ishii 1992). This finding indicates that the improvements of leaf photosynthesis have occurred with the advance of breeding high-yielding cultivars. However, the cause or mechanism of the improvements is still not clear.

In our study, P_N in the hybrid rice combinations Peiai 64S/E32 (with a higher yield potential) and Shanyou 63 (as a reference) was measured at the panicle differentiation stage. Moreover, some related parameters including carboxylation efficiency (CE), quantum yield (Φ_c), compensation irradiance (I_c), photorespiration rate (R_P), and chlorophyll (Chl) fluorescence were compared.

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Abbreviations: CE – carboxylation efficiency; Chl – chlorophyll; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; F_v/F_m – potential photochemical efficiency of photosystem 2 measured with adequately dark-adapted leaves; $\Delta F/F_m$' – actual photochemical efficiency of PS2 measured with irradiated leaves; I_c – compensation irradiance; LMA – leaf mass per area; LT – leaf thickness; PPFD – photosynthetic photon flux density; P_N – net photosynthetic rate; PS – photosystem; q_N – non-photochemical quenching; R_D – dark respiration rate; R_P – photorespiration rate; Φ_c – apparent quantum yield of CO_2 assimilation; Γ – compensation CO_2 concentration.

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Materials and methods

Plants: Seeds of rice (*Oryza sativa* L. hybrid combinations Shanyou 63 and Peiai 64S/E32) were sown in plastic pots containing garden soil on May 5, 2000. The pots were placed in the field of Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences. Plants of both hybrid combinations were grown in the same pot. In order to obtain uniform plants, the seedlings of each combination were thinned from 7-9 to 4 per pot after the appearance of the second leaf. Soybean cakes were used as base fertiliser for the plants. All the measurements were performed with the first or second fully expanded, disease-free leaves counted from the top of the plant at the panicle differentiation stage (from July 1 to 27, 2000).

Gas exchange: Leaf P_N was measured using a portable Infrared Gas Analysis System *CI-301* (*CID*, Vancouver, U.S.A.) on attached leaves in the laboratory between 11:00 and 12:00 (Beijing time). A halogen lamp provided irradiation, with a flowing water layer to remove heat. Gas-exchange measurements were performed at a saturating PPFD of about 1 200 μmol(photon) m^{-2} s^{-1}, air CO_2 concentration of 380-415 cm^3 m^{-3}, and air temperature of 31-34 °C after the end of the induction period of photosynthesis. Then some leaves were used to measure the photosynthetic response to PPFD, Φ_c (Xu *et al.* 1987), and CE (Caemmerer and Farquhar 1981). I_c and dark respiration rate (R_D) were obtained from the P_N *vs.* PPFD curve. Similarly, CO_2 compensation concentration (Γ) and R_P were obtained from the P_N *vs.* C_i curve.

Leaf thickness (LT), leaf dry mass/area (LMA), and leaf Chl content: Following P_N measurements, some leaves were taken for these determinations. Every leaf was cut into 20 pieces and these pieces were piled up. Then the thickness of leaf was obtained by measuring the pile by vernier callipers. Leaf segments with same area were killed at 100 °C for 1 h and then dried at 65 °C till constant mass to obtain LMA (leaf dry mass/area). The Chl content of leaf was determined spectrophotometrically according to Arnon (1949).

Chl *a* fluorescence was measured with a portable *PAM-2000* fluorimeter (*H. Walz*, Effeltrich, Germany) with the standard settings at room temperature (28 °C). After the measurements of P_N, the leaves were put in the dark for 2 h. After the measurements of potential photochemical efficiency (F_v/F_m), the leaves were irradiated at a PPFD of 1 000 μmol m^{-2} s^{-1} for 1 h. Then the actual photochemical efficiency ($\Delta F/F_m'$) and non-photosynthetic quenching (q_N) were measured. The calculations were made according to Genty *et al.* (1989) and van Kooten and Snel (1990).

Differences between the two hybrid combinations in all measured parameters were tested for significance by a Student's *t*-test. The levels of significance were $p<0.05$ and $p<0.01$, respectively.

Results

P_N and apparent quantum yield: The patterns of leaf photosynthetic response to irradiance were similar and the saturating PPFDs were almost the same [about 1 000 μmol(photon) m^{-2} s^{-1}] in the two hybrid combinations

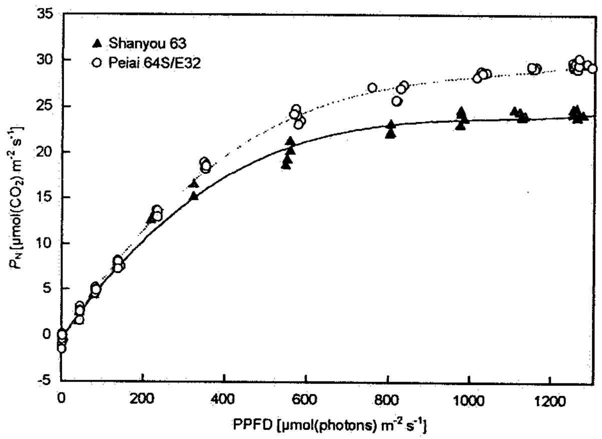

Fig. 1. Response of net photosynthetic rate (P_N) to photosynthetic photon flux density (PPFD) of leaves in the hybrid combinations Shanyou 63 and Peiai 64S/E32 at the panicle differentiation stage. P_N was measured by *CI-301* at 455 cm^3 m^{-3} CO_2 and 32 °C in the laboratory on July 25, 2000. Only one of five repeats is listed here.

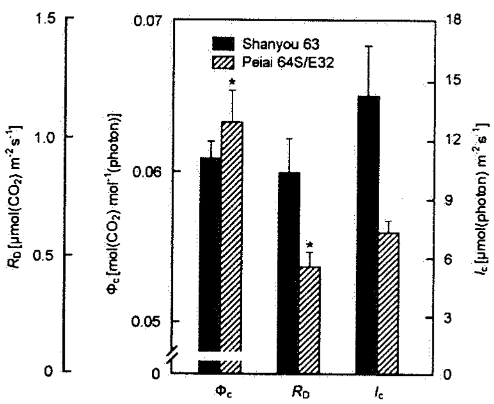

Fig. 2. Apparent quantum yield of carbon assimilation (Φ_c), dark respiration rate (R_D), and compensation irradiance (I_c) of leaves in the hybrid rice combinations Shanyou 63 and Peiai 64S/E32 at the panicle differentiation stage. Means of 5 leaves ± SE (*bars*). *$p<0.05$. The first fully expanded leaf was measured by CI-301 at 30-32 °C in the laboratory on July 23-27, 2000.

(Fig. 1). However, Peiai 64S/E32 had a significantly higher (6-26 %) P_N at saturating PPFD (Table 1), a significantly higher Φ_c at low irradiance, a significantly lower R_D, and a lower I_c than Shanyou 63 (Fig. 2). Shanyou 63 had a lower g_s but a higher C_i than Peiai 64S/E32 (Table 1).

PS2 photochemical efficiency: Peiai 64S/E32 showed a slightly higher PS2 photochemical efficiency (F_v/F_m and $\Delta F/F_m$') and a slightly lower non-photosynthetic quenching (q_N) than Shanyou 63 (Table 2).

Carboxylation efficiency of Peiai 64S/E32 was much higher than that of Shanyou 63, and the difference was significant (Table 3). Peiai 64S/E32 also had a significantly higher R_P than Shanyou 63. However, there was no significant difference in the CO_2 compensation concentration between the two hybrid combinations (Table 3).

LT, LMA, leaf Chl content: Leaf Chl content of Peiai 64S/E32 was significantly greater than that of Shanyou 63. Moreover, Peiai 64S/E32 had a larger leaf thickness and leaf dry mass/area than Shanyou 63, but these differences were not always significant (Table 4).

Table 1. Net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], stomatal conductance, g_s [mol(H_2O) m^{-2} s^{-1}], and intercellular CO_2 concentration. C_i [cm^3 m^{-3}] of leaves in the hybrid rice combinations Shanyou 63 and Peiai 64S/E32 grown in pots at the panicle differentiation stage. Means ± SE of 3-6 leaves. $^*p < 0.05$, $^{**}p < 0.01$. The first or second fully expanded leaves were measured by *CI-301* in the laboratory during 11:00-12:00 on July 1-27, 2000. After the end of the induction period of photosynthesis, P_N was measured at saturated PPFD of about 1 200 μmol(photon) m^{-2} s^{-1}, air CO_2 concentration 380-415 cm^3 m^{-3}, and air temperature 31-34 °C.

	Shanyou 63			Peiai 64S/E32		
	P_N	g_s	C_i	P_N	g_s	C_i
July 1	26.8±1.2	0.193±0.011	319.3±1.4	30.7±0.6*	0.203±0.016*	311.0±10.8
July 6	22.8±1.1	0.172±0.011	247.4±3.9	27.5±1.3*	0.187±0.014	231.1±3.2
July 7	24.0±1.3	0.170±0.014	248.6±6.6	27.8±0.9	0.190±0.007	243.5±5.9
July 8	23.0±1.5	0.170±0.008	226.2±5.0	25.2±0.3	0.169±0.007	209.7±8.4
July 9	24.8±0.8	0.188±0.007	240.4±3.9	28.4±1.0*	0.202±0.011	232.1±7.4
July 16	22.6±0.7	0.256±0.003	252.4±7.9	28.5±0.3**	0.279±0.011	231.1±1.0
July 17	23.4±0.5	0.247±0.024	245.9±10.0	27.6±0.2**	0.293±0.021	249.7±5.7
July 18	25.3±0.5	0.246±0.029	267.9±4.4	31.1±0.6**	0.201±0.052	171.4±58.3
July 23	24.7±3.5	0.339±0.182	281.9±68.0	27.0±3.0	0.245±0.045	257.7±7.2
July 27	21.8±0.7	0.268±0.014	285.7±8.1	23.2±0.5	0.268±0.008	278.9±4.9

Table 2. Chlorophyll fluorescence parameters of leaves in the hybrid rice combinations Shanyou 63 and Peiai 64S/E32 grown in pots at the panicle differentiation stage. Means ± SE of 4-5 leaves. $^*p < 0.05$. The first or second fully expanded leaves were measured by *PAM-2000* in the laboratory (room temperature 28 °C) on July 1-9, 2000. After the measurement of P_N, the leaves were put in the dark for 2 h. Following the determination of potential photochemical efficiency (F_v/F_m), the leaves were irradiated at 1 000 μmol(photon) m^{-2} s^{-1} for 1 h. Then the actual photochemical efficiency ($\Delta F/F_m$') and non-photochemical quenching (q_N) were measured.

	Shanyou 63			Peiai 64S/E32		
	F_v/F_m	$\Delta F/F_m$'	q_N	F_v/F_m	$\Delta F/F_m$'	q_N
July 1	0.773±0.009	-	-	0.780±0.008	-	-
July 3	0.799±0.012	0.509±0.027	0.244±0.037	0.821±0-006	0.527±0.004	0.218±0.003
July 6	0.800±0.010	0.445±0.006	0.174±0.013	0.832±0.004*	0.481±0.014	0.171±0.010
July 8	0.817±0.006	0.421±0.006	0.154±0.004	0.825±0.008	0.473±0.034	0.183±0.028

Table 3. Carboxylation efficiency, CE [mol(CO_2) m^{-2} s^{-1}], photorespiration rate, R_P [μmol(CO_2) m^{-2} s^{-1}], and CO_2 compensation concentration, Γ [cm^3 m^{-3}] of leaves in the hybrid rice combinations Shanyou 63 and Peiai 64S/E32 grown in pots at the panicle differentiation stage. Means ± SE of 3 leaves, 3 repetitions. $^*p < 0.05$, $^{**}p < 0.01$. The first fully expanded leaves were measured by *CI-301* at 30-32 °C in the laboratory on July 16-18, 2000.

	Shanyou 63			Peiai 64S/E32		
	1	2	3	1	2	3
CE	0.1533±0.0069	0.1516±0.0101	0.1660±0.0202	0.2014±0.0016**	0.1893±0.0031*	0.2676±0.0258*
R_P	8.4±0.2	8.8±0.4	9.2±0.4	11.4±0.4**	11.3±0.4*	14.6±0.8*
Γ	55.4±3.1	58.1±2.7	53.3±5.1	56.4±2.4	59.8±3.1	55.0±2.4

Table 4. Leaf thickness, LT [µm]), leaf dry mass/area, LMA [g m^{-2}], and leaf Chl content [g m^{-2}] in the hybrid rice combinations Shanyou 63 and Peiai 64S/E32 grown in pots at the panicle differentiation stage. Five repetitions, means of 4-7 leaves ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$. The first and second fully expanded leaves were used in the measurements.

	Shanyou 63					Peiai 64S/E32				
	1	2	3	4	5	1	2	3	4	5
LT	88.9±4.0	81.7±2.2	78.3±5.0	76.9±1.3	69.4±1.9	95.6±1.1	94.2±4.6	92.5±7.5	84.1±2.3*	85.6±1.1**
LMA	25.93±0.04	26.30±1.17	30.00±0.14	29.84±1.48	-	27.74±1.21	28.14±1.54	32.25±1.25	34.47±0.68*	-
Chl	0.534±0.018	-	-	-	-	0.616±0.010**	-	-	-	-

Discussion

There are two possible factors leading to the significant difference in P_N between Peiai 64S/E32 and Shanyou 63, namely stomatal and non-stomatal or mesophyll factor. A higher C_i in Shanyou 63 indicates that the main cause of its lower P_N is its lower photosynthetic capacity of mesophyll cells rather than its lower stomatal conductance (Table 1). In other words, the higher P_N in Peiai 64S/E32 is mainly due to the higher photosynthetic capacity of its mesophyll cells. Our result is similar to those obtained in other crops, which indicates that the difference in P_N among interspecies and/or intraspecies do not mainly arise from altered stomatal limitations to photosynthesis (Johnson *et al.* 1987, Cornish *et al.* 1991, Jiang and Xu 2000).

The higher photosynthetic capacity of mesophyll cells in Peiai 64S/E32 may be explained by its higher amount and/or activity of RuBPC. Some studies have showed that P_N is positively correlated to the activity and/or amounts of RuBPC (Hesketh *et al.* 1981, Evans 1986). *In vivo* carboxylation efficiency (CE), the slope of the initial linear portion of the P_N *vs.* C_i curve, is usually considered as an index of amount of activated RuBPC in leaf (Caemmerer and Farquhar 1981). The significantly higher CE in Peiai 64S/E32 (Table 3) indicates that it has a higher amount of activated RuBPC in leaves than Shanyou 63. Therefore, in the new hybrid combination, the increase in amount of RuBPC is possibly an important factor leading to its higher P_N.

In correspondence with its greater amount of RuBPC, Peiai 64S/E32 with a higher P_N has also a significantly higher R_P (Table 3). It is not surprising because the substrate of photorespiration, phosphoglycollate, is from a reaction catalysed also by RuBP oxygenation, which occurs simultaneously with RuBP carboxylation by the same enzyme. Thus, photorespiration is an inevitable consequence of RuBPCO operation (Miziorko and Lorimer 1983). A positive correlation between P_N and R_P was actually found (Lloyd and Canvin 1977). Moreover, Aliev *et al.* (1996) reported that the activities of both RuBP oxygenation and RuBP carboxylation were larger in highly productive wheat genotypes than in the low productive ones. Our results about P_N and R_P in the hybrid rices (Tables 1 and 3) are consistent with their findings. It is probably impossible to obtain a new cultivar with both high P_N and low R_p if RuBPCO is not transformed by genetic engineering.

The greater P_N in Peiai 64S/E32 can be partly attributed to its lower R_D (Fig. 2). Since P_N is a result of subtracting R_D from rough photosynthesis, a decline in R_D must lead to an increase in P_N. Carbon loss through dark respiration can affect the difference in P_N (Hanson 1992). Therefore, a lower R_D may be selected as a good character when a new cultivar with high P_N is bred to increase crop yield.

Photosynthetic performance in Peiai 64S/E32 is better not only at high irradiance, as shown by the higher P_N at saturating irradiance (Table 1 and Fig. 1), but also at low irradiance, as shown by a higher Φ_c and a lower I_c (Fig. 2). Its higher Φ_c and lower I_c indicate that Peiai 64S/E32 is more effective in the utilisation of low irradiance than Shanyou 63. These characters are very beneficial to gain high crop yield because most leaves of plants in canopy are exposed to low irradiance for long time. Actually, a significantly positive correlation between Φ_c and yield has been reported in *Hevea* (Nugawela *et al.* 1995). However, its higher Φ_c does not imply a higher efficiency of energy conversion in Peiai 64S/E32 since Φ_c is apparent but not actual quantum yield. Perhaps, the higher Φ_c in Peiai 64S/E32 results from a higher Chl content (Table 4). The result showing that there is no significant difference in the photochemical efficiency of PS2 (Table 2) between Peiai 64S/E32 and Shanyou 63 supports the deduction. Our results that Peiai 64S/E32 had a significantly higher Φ_c but lower R_D and I_c than Shanyou 63 are consistent with those of Jiang *et al.* (2000), but inconsistent with those of Barro *et al.* (1996). Jiang *et al.* (2000) reported that, in 5 representative wheat cultivars with different yield potential, the higher yield cultivars had higher P_N and Φ_c, but lower I_c and R_D. Faville *et al.* (1999) found that the high-yielding cultivar, Korapiro, tended to have a high Φ_c and low I_c. These cultivars also seem to assimilate CO_2 at a higher rate under lower PPFD and to consume less carbon through dark respiration.

Altogether, the new hybrid rice Peiai 64S/E32 possesses some better characters in photosynthesis and some related physiological processes than hybrid rice Shanyou 63. Besides the good plant type and canopy structure, the higher P_N, Φ_c, and lower I_c and R_D in leaves of Peiai 64S/E32 also contribute to its higher efficiency of photon utilisation and better yield.

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