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The relationships between carbon isotope discrimination and photosynthesis and rice yield under shading

L. Wang[a,1], F. Deng[a,1], T.Q. Lu[b], M. Zhao[a], Sh.L. Pu[a], Sh.X. Li[a], W.J. Ren[a,*]

[a]Key Laboratory of Crop Ecophysiology and Farming System in Southwest China, Ministry of Agriculture, Sichuan Agricultural University, Chengdu 611130, China.
[b]Mianyang Institute of Agricultural Sciences, Mianyang 621023, China.
[1]These authors contributed equally to this work.
*Corresponding author. E-mail: rwjun@126.com

Abstract

The measurement of carbon isotope discrimination (Δ) provides an integrated insight into the response of plants to environmental change. To investigate the potential use of Δ for identifying shade tolerance in rice, five rice varieties were selected and artificially shaded (53% light reduction) during the grain-filling period in 2010 and 2011, in Sichuan, China. Shading treatment had a significant influence on the Δ of rice organs, resulting in clear increases in the Δ of stems plus sheaths (ΔS_m), rice grains (ΔG_m) and rice flour (ΔF_m) at maturity, but a reduction in the Δ of leaves (ΔL_g) at the grain-filling stage. The relationships between Δ and leaf photosynthetic and chlorophyll fluorescence characteristics and grain yield showed a close dependence on plant organs and light regimes. Under shading treatment, photosynthetic rate was negatively associated with ΔG_m and the Δ of stems plus sheaths at the grain-filling stage (ΔS_g), whereas ΔS_m was significantly (P<0.05) negatively correlated with the quantum yield of PSII (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ). Moreover, grain filling and grain weight under shading treatment were positively correlated with ΔS_g, but negatively related to ΔS_m in 2011. In contrast, a significantly (P<0.01) negative association between grain weight and ΔL_g was observed in 2010. It was found that lower values of ΔL_g, ΔS_m and ΔG_m in rice indicated better light-harvesting and light-use capability and also higher grain filling and grain weight of rice.

Keywords: Carbon isotope discrimination; Light regime; Photosynthesis; Rice.

Abbreviations: Ca: atmospheric CO_2 concentration; C_i: intercellular CO_2 concentration; Cond: conductance to H_2O; F_v/F_m: maximum efficiency of PSII photochemistry under dark-adaption; NPQ: non-photochemical quenching; P_n: photosynthetic rate; qP: photochemical quenching; Tr: transpiration rate; Φ_{PSII}: quantum yield of PSII; Δ: carbon isotope discrimination; ΔF_m: Δ of rice flour at maturity; ΔG_m: Δ of rice grain at maturity; ΔL_g: Δ of leaves at the grain-filling stage; ΔS_g: Δ of stem plus sheath at the grain-filling stage; ΔS_m: Δ of stem plus sheath at maturity.

Introduction

It is predicted that by 2050, the world's population will reach 9 billion and thus ensuring food security will become one of this century's largest challenges (Godfray et al., 2010; Zhao et al., 2013). As one of the three major cereal crops, rice (*Oryza sativa* L.) accounts for more than 40% of the world's food production (Makino, 2011) and

supports more than half of the Chinese population (Jian et al., 2014). However, production of the crop has suffered from unfavorable conditions, such as shading caused by environmental change and industrial development (Li et al., 2010; Wang et al., 2015).

Solar radiation, one of the most dynamic environmental factors, is the principal source of energy for photoautotrophic plants (Kubásek et al., 2013). Under shading treatment, the photosynthetically active radiation that plants capture for photosynthesis and the intensity of solar radiation that determines the level of photosynthetically active radiation are reduced (Mo et al., 2015), thereby altering the morphology, physiology, yield and quality of plants (Li et al., 2010; Wang et al., 2013; Mo et al., 2015; Wang et al., 2015). Numerous studies have been conducted in an attempt to understand how plants respond to shading treatment. For example, Li et al. (2010) have reported that shading increased the leaf area index, length of peduncle internodes and upper leaf area of wheat (*Triticum aestivum*). Shading also increased the amount of chlorophyll and xanthophyll pigments, as well as the superoxide dismutase and peroxidase activities of leaves (Zheng et al., 2011). Mu et al. (2010) and Mauro et al. (2011) found that shading increased the maximum efficiency of PSII photochemistry under dark adaption (F_v/F_m) and intercellular CO_2 concentration (C_i), but reduced photosynthetic rate and plant dry weight. Shading also caused a reduction in grain yield by reducing grain filling and grain weight (Mu et al., 2010; Mo et al., 2015; Wang et al., 2015), as well as affecting grain quality, such as increasing protein content and altering starch pasting viscosity (Wang et al., 2013; Mo et al., 2015).

To minimize the damage of shading on crop production, there is an urgent need for highly shade-tolerant varieties. However, the conventional identification of shade tolerance in plants is based on field observations of plant performance and investigation of plant growth (Kennedy et al., 2006), which are often destructive, labor- and time-consuming and limited in their application to breeding programs (Merah et al., 2001). The use of chlorophyll fluorescence and photosynthesis measurement systems has provided a non-destructive means for assessing the shade tolerance of plants (Mu et al., 2010). For instance, by using such systems, we have demonstrated that shade-tolerant rice varieties are associated with better light harvesting and light-use efficiency and 'IIyou 498', which has an increased F_v/F_m and quantum yield of PSII (Φ_{PSII}) but decreased non-photochemical quenching (NPQ), has been selected as a highly shade-tolerant variety (Wang et al., 2015). Nevertheless, the measurements of chlorophyll fluorescence and photosynthesis characteristics are often limited by weather and are difficult to integrate over time (Kennedy et al., 2006). Therefore, more convenient and feasible means are needed to identify shade tolerance in plants.

In nature, there are two stable isotopes of carbon, ^{12}C and ^{13}C. During photosynthesis, plants discriminate against ^{13}C, which results in a depletion of ^{13}C in plant dry matter (Farquhar and Richards, 1984; Merah et al., 2001). Carbon isotope discrimination (a measure of the $^{13}C{:}^{12}C$ ratio in plants vs. the same ratio in the atmosphere, Δ) (Farquhar and Richards, 1984; Körner et al., 1988), which is closely related to the grain or biomass production of plants (Ngugi et al., 1996) and primarily reveals the balance between CO_2 supply and demand in plants (Hussain et al., 2011), can reflect the physical, chemical and metabolic processes of plants involved in carbon transformations (Farquhar et al., 1989; Scartazza et al., 1998). Therefore, Δ is routinely used to infer the growth, respiration, photosynthesis and water-use efficiency of plants (Farquhar et al., 1989; Scartazza et al., 1998; Damesin and Lelarge, 2003; McDowell and Sevanto, 2010; Misra et al., 2010), as well as the net ecosystem CO_2 fluxes between photosynthesis and respiration (Zobitz et al., 2008).

The measurement of Δ provides an effective means by which to study the response of plants to environmental changes, such as variable water, fertilizer and atmospheric CO_2 supplies (Fravolini et al., 2002; Damesin and Lelarge, 2003; Misra et al., 2010;

Moghaddam et al., 2013). Furthermore, Kennedy et al. (2006) have demonstrated the potential use of Δ for the selection of shade-tolerant species. However, our understanding of Δ with regard to identifying shade tolerance in rice is limited. Nevertheless, whereas most studies on Δ have focused on the leaf level (Williams et al., 2001; Kromdijk et al., 2008; Hussain et al., 2011), Moghaddam et al. (2013) have indicated that the study of Δ in different plant organs might assist agronomists in improving the stability of plants in changeable climates.

In Sichuan Basin, there is less than 1200 hours of annual sunshine and the total annual solar radiation ranges from 3345 to 3763 MJ m^{-2}, which leads to a reduction in the yield and quality of crops (Huang, 1998; Wang et al., 2013; Yang et al., 2013). The specific objectives of this study were to (1) investigate the effect of shading treatment on the Δ of rice organs; (2) assess the relationship between Δ and the chlorophyll fluorescence and photosynthesis characteristics of leaves and also grain yield and its components and (3) evaluate the potential use of Δ for the identification of shade-tolerant rice varieties.

Materials and Methods

Experimental design

The experimental details have been described in Wang et al. (2013 and 2015). In brief, the experiment was conducted in randomized block design to study the effects of shading on different rice varieties (IIyou 498, Gangyou 188, Dexiang 4103, Gangyou 527 and Chuanxiang 9838), with three replications, on the farm of Sichuan Agricultural University, Ya'an (29° 58′ N and 102° 59′ E), Sichuan Province, China, in 2010–2011. On 20 May 2010 and 25 May 2011, the 50-day old seedlings were transplanted at a spacing of 33.3 cm × 20.0 cm with two plants per hill. 180 kg hm^{-2} of Na, 90 kg hm^{-2} of P_2O_5 and 180 kg hm^{-2} of K_2O were applied. The high efficiency irrigation described by Wang et al. (2013) was used.

Shading treatment was implemented from heading (August 5, 2010) to maturity (September 26, 2010) and from heading (August 9, 2011) to 30 d after heading (September 7, 2011). A single layer of white cotton yarn screen was placed 2-m above the rice canopy to create a 53% shading environment. The control (CK) group plants were grown under full sunlight.

Sampling and measurements

Carbon isotope discrimination

Carbon isotope discrimination (Δ) was analyzed in rice leaves and stems plus sheaths at the grain-filling stage (on day 20 of shading treatment, 2010–2011), as well as in rice grains (2010) and rice flour and stems plus sheaths (2011) at maturity. In each experimental plot, plants from hills were harvested at the grain-filling and maturity stages. Rice leaf and stem plus sheath samples were oven-dried at 105 °C for 1 h and then at 80 °C until a constant weight was obtained. Rice seeds were dried at room temperature for approximately 3 months and then shelled to mill rice for rice flour production. The dried samples were ground using a CT410 mill (FOSS SCINO Co., Ltd, China) and sifted through a 0.5-mm screen. Carbon isotope discrimination was performed using an Isotope Ratio Mass Spectrometer (Thermo Delta V advantage, Agawam, USA) in the Chinese Academy of Forestry, Beijing. The results were expressed as $\delta^{13}C$ (‰) = [($^{13}C/^{12}C$)$_{sample}$ - ($^{13}C/^{12}C$)$_{PDB}$] × 1000/($^{13}C/^{12}C$)$_{PDB}$ (1). A secondary standard, potato starch, calibrated against Pee Dee Belemnite (PDB)

carbonates was used for comparison. Δ was calculated (Farquhar et al., 1989) as Δ (‰) = $[(\delta^{13}C_{air} - \delta^{13}C_{sample})/(1 + \delta^{13}C_{sample})] \times 1000$ (2), where $\delta^{13}C_{air}$ has a current deviation of approximately -7.7‰. Carbon isotope discrimination in rice leaves and stems plus sheaths at the grain-filling stage and stems plus sheaths, grain and flour at maturity, are referred to as ΔL_g, ΔS_g, ΔS_m, ΔG_m and ΔF_m, respectively.

Photosynthetic and chlorophyll fluorescence characters

Photosynthetic parameters (2010–2011) and chlorophyll fluorescence (2011) of the flag leaf after 20 d of shading were determined using an LI-6400XT Portable Photosynthesis System (LI-COR, Inc., USA) and a MINI-PAM Photosynthesis Yield Analyzer (Heinz Walz GmbH, Germany), respectively. The measuring conditions and calculation methods have been described in Wang et al. (2015).

Yield and yield components

At maturity, five plants from each plot were harvested and threshed to determine grain filling and grain weight, while grain yield was recorded from a 7-m^2 area in each plot by adjusting to a standard moisture content of 13.5%.

Statistical analysis

The experiment was a 5×2 factorial randomized block design. The data were subjected to two-way analysis of variance (ANOVA) and Fisher's protected least significance difference (LSD) test was used to determine the significant difference between treatments (SPSS 16.0). Data represented in the tables are the means ± standard deviations (SD) of three replicates for each treatment.

Results

Effect of shading treatment on carbon isotope discrimination

There were marked effects of shading treatment and rice varieties on the carbon isotope discrimination (Δ) of rice organs (Table 1). Even though we observed no significant effect of shading treatment on the Δ of leaves at the grain-filling stage (ΔL_g) and the Δ of stem plus sheath at the grain-filling stage (ΔS_g) in 2010, shading treatment significantly (P<0.05) reduced the ΔL_g but increased the ΔS_g in 2011. Furthermore, shading treatment significantly (P<0.05) increased the Δ of rice grain at maturity (ΔG_m) in 2010 and the Δ of stem plus sheath at maturity (ΔS_m) and the Δ of rice flour at maturity (ΔF_m) in 2011, which resulted in increases of 1.62%, 1.16% and 1.61%, respectively.

Furthermore, the Δ of rice organs was significantly (P<0.01) affected by the interaction of shading treatment and rice varieties. Compared to full sunlight (control), shading treatment reduced the ΔL_g of IIyou 498, Dexiang 4103 and Chuanxiang 9838, but increased that of Gangyou 188 and Gangyou 527. However, significant differences (P<0.05) were only obtained in IIyou 498, Gangyou 188 and Chuanxiang 9838. Shading treatment also significantly (P<0.05) improved the ΔS_g of Gangyou188 in both years and the ΔS_g of IIyou 498 and Gangyou 527 in 2011, but markedly decreased the ΔS_g of Chuanxiang 9838 in 2010. With shading treatment, there was a significant (P<0.05) increase in the ΔG_m, ΔS_m and ΔF_m of IIyou 498, Dexiang 4103 and Chuanxiang 9838, but no significant influence on the ΔG_m of Gangyou 527, ΔS_m of Gangyou 188 and Gangyou 527 and the ΔF_m of Gangyou 188.

Table 1. Carbon isotope discrimination in rice leaves (ΔL_g) and stems plus sheaths (ΔS_g) at the grain-filling stage and also stems plus sheaths (ΔS_m), rice grains (ΔG_m) and rice flour (ΔF_m) at maturity subjected to shading treatment in 2010–2011.

Year	2010			2011			
Treatment	ΔL_g(‰)	ΔS_g(‰)	ΔG_m(‰)	ΔL_g(‰)	ΔS_g(‰)	ΔS_m(‰)	ΔF_m(‰)
Full sunlight							
Ilyou 498	21.53 ± 0.07[bc]	21.64 ± 0.05[bc]	20.83 ± 0.04[e]	20.51 ± 0.04[b]	20.65 ± 0.03[cd]	20.63 ± 0.08[d]	19.39 ± 0.12[e]
Gangyou 188	21.52 ± 0.08[bc]	21.60 ± 0.04[bcd]	20.70 ± 0.07[f]	20.51 ± 0.06[b]	20.63 ± 0.09[d]	20.86 ± 0.06[c]	19.99 ± 0.13[cd]
Dexiang 4103	21.47 ± 0.07[c]	21.70 ± 0.04[ab]	21.02 ± 0.09[d]	20.65 ± 0.09[a]	20.69 ± 0.16[bcd]	20.67 ± 0.07[d]	19.94 ± 0.09[cd]
Gangyou 527	21.59 ± 0.02[b]	21.62 ± 0.06[bc]	21.29 ± 0.06[abc]	20.17 ± 0.07[c]	20.31 ± 0.10[e]	21.01 ± 0.02[b]	20.11 ± 0.14[bc]
Chuanxiang 9838	21.47 ± 0.06[c]	21.72 ± 0.03[ab]	20.83 ± 0.08[e]	20.67 ± 0.05[a]	20.81 ± 0.18[abc]	20.65 ± 0.03[d]	19.98 ± 0.10[cd]
Mean	21.52	21.66	20.93	20.50	20.62	20.76	19.88
Shading							
Ilyou 498	21.34 ± 0.08[d]	21.49 ± 0.12[cd]	21.17 ± 0.02[c]	20.24 ± 0.05[c]	20.88 ± 0.07[a]	20.85 ± 0.12[c]	20.20 ± 0.05[b]
Gangyou 188	21.69 ± 0.04[a]	21.84 ± 0.08[a]	21.26 ± 0.14[bc]	20.72 ± 0.06[a]	20.80 ± 0.01[abc]	20.89 ± 0.03[c]	19.83 ± 0.16[d]
Dexiang 4103	21.46 ± 0.03[c]	21.71 ± 0.01[ab]	21.35 ± 0.02[ab]	20.46 ± 0.02[b]	20.85 ± 0.13[ab]	20.88 ± 0.04[c]	20.25 ± 0.10[b]
Gangyou 527	21.75 ± 0.05[a]	21.70 ± 0.20[ab]	21.40 ± 0.06[a]	20.20 ± 0.07[c]	20.67 ± 0.03[cd]	21.11 ± 0.01[b]	20.47 ± 0.17[a]
Chuanxiang 9838	21.35 ± 0.02[d]	21.45 ± 0.14[d]	21.19 ± 0.04[c]	20.46 ± 0.12[b]	20.68 ± 0.03[bcd]	21.28 ± 0.05[a]	20.23 ± 0.14[b]
Mean	21.52	21.63	21.27	20.42	20.78	21.00	20.20
L	0.016	0.356	166.02**	15.93**	18.79**	111.65**	51.76**
V	27.54**	3.36*	28.95**	54.20**	7.97**	28.71**	15.61**
L × V	13.18**	6.73**	7.28**	17.93**	4.81**	21.55**	12.62**

L, light regime; V, variety. Values are means ± SD of three independent replications (n=3). Different letters within the same column indicate statistically significant differences between treatments, according to Fisher's protected least significance differences test at the 5% level. * $P<0.05$, ** $P<0.01$.

Effect of shading treatment on photosynthetic characteristics

The photosynthetic rate (P_n), conductance to H_2O (Cond), intercellular CO_2 concentration (C_i) and transpiration rate (Tr) of rice flag leaf at the grain-filling stage were significantly ($P<0.01$) affected by shading treatment (except C_i in 2011), rice varieties and their interaction in 2010 and 2011 (Table 2). Shading treatment, compared to the control, significantly ($P<0.05$) reduced the P_n and Cond of flag leaf in 2010 and 2011, as well as the Tr of flag leaf in 2010. Furthermore, the C_i in 2010 and Tr in 2011 were clearly reduced by shading treatment. Under shading treatment, the P_n of the five varieties was significantly ($P<0.05$) decreased, which resulted in a 5.30% to 19.86% and 3.04% to 13.91% reduction in P_n in 2010 and 2011, respectively (Wang et al., 2015). Shading treatment also reduced the Cond of the flag leaf of IIyou 498, Dexiang 4103 and Chuanxiang 9838, but increased that of the flag leaf of Gangyou 527 in both years. Shading treatment markedly ($P<0.05$) improved the C_i of IIyou 498, Gangyou 188 and Dexiang 4103 in 2010 and also that of Gangyou 188 in 2011. However, a significant ($P<0.05$) reduction in the C_i of Dexiang 4103 and Chuanxiang 9838 was observed in 2011. Furthermore, shading treatment significantly ($P<0.05$) increased the Tr of IIyou 498, but reduced that of Dexiang 4103 in both 2010 and 2011.

Relationship between photosynthetic characteristics and carbon isotope discrimination

A highly considerable correlation was noted between photosynthetic characteristics and carbon isotope discrimination across or within light regimes in both years (Table 3). The P_n was significantly ($P<0.01$ or $P<0.001$) negatively associated with ΔG_m in 2010 and with ΔS_m and ΔF_m in 2011. A significantly ($P<0.05$) or highly significantly ($P<0.01$) negative correlation was found between P_n and ΔS_g and ΔG_m under shading treatment in 2010 and ΔS_m and ΔF_m under control conditions in 2011. In contrast, a significantly ($P<0.05$) positive relationship was observed between P_n and ΔS_m under shading treatment in 2011. No significant relationship was found between Cond and the carbon isotope discrimination of organs either across or within light regimes in 2010. However, Cond was significantly ($P<0.05$) positively related to ΔL_g across light regimes and also to ΔL_g and ΔS_g ($P<0.01$) under control conditions, but was markedly ($P<0.001$) negatively associated with ΔS_m across light regimes and under control conditions and with ΔS_g under shading in 2011. There were significantly ($P<0.001$) negative correlations between C_i and ΔS_m under control conditions and across light regimes in 2011, whereas C_i was significantly positively related to ΔG_m (2010, $P<0.01$ or $P<0.001$) and ΔL_g (2011, $P<0.05$) across light regimes or under control conditions. Furthermore, Tr was markedly ($P<0.05$) negatively associated with ΔS_g across or within light regimes, but positively related to ΔL_g and ΔG_m under control conditions in 2010.

Table 2. Photosynthetic rate (P_n, μmol CO_2 m^{-2} s^{-1}) (Wang et al., 2015), conductance to H_2O (Cond, mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (C_i, μmol CO_2 mol^{-1}) and transpiration rate (Tr, mmol H_2O m^{-2} s^{-1}) in rice flag leaf at the grain-filling stage subjected to shading treatment in 2010–2011.

Year		2010				2011			
Treatment		P_n	Cond	C_i	Tr	P_n	Cond	C_i	Tr
Full sunlight									
Iiyou 498		14.29 ± 0.04[d]	0.492 ± 0.011[bc]	284.61 ± 4.56[e]	8.78 ± 0.11[b]	26.38 ± 0.23[a]	1.307 ± 0.085[c]	328.17 ± 2.86[bc]	9.21 ± 0.12[d]
Gangyou 188		14.07 ± 0.06[b]	0.421 ± 0.013[de]	289.49 ± 1.41[e]	8.72 ± 0.31[bc]	25.65 ± 0.34[c]	1.053 ± 0.095[e]	319.50 ± 1.78[d]	9.68 ± 0.36[c]
Dexiang 4103		14.10 ± 0.12[ab]	0.553 ± 0.044[a]	299.40 ± 6.19[cd]	8.41 ± 0.11[cd]	26.02 ± 0.08[b]	1.421 ± 0.027[b]	333.06 ± 0.43[ab]	8.84 ± 0.03[e]
Gangyou 527		13.79 ± 0.24[c]	0.480 ± 0.042[bc]	305.85 ± 6.28[bc]	9.32 ± 0.07[a]	24.22 ± 0.15[e]	1.023 ± 0.074[e]	322.73 ± 3.19[cd]	9.27 ± 0.05[d]
Chuanxiang 9838		13.58 ± 0.11[c]	0.482 ± 0.013[bc]	302.35 ± 2.52[cd]	8.33 ± 0.16[de]	24.68 ± 0.19[d]	1.547 ± 0.045[a]	335.35 ± 4.24[a]	8.10 ± 0.07[f]
Mean		13.97	0.486	296.34	8.71	25.39	1.270	327.76	9.02
Shading									
Iiyou 498		13.08 ± 0.12[d]	0.481 ± 0.008[bc]	306.45 ± 1.61[bc]	9.26 ± 0.12[a]	23.09 ± 0.10[f]	1.030 ± 0.088[e]	325.12 ± 4.50[cd]	10.31 ± 0.17[a]
Gangyou 188		12.12 ± 0.04[e]	0.411 ± 0.011[e]	297.36 ± 3.52[d]	7.63 ± 0.25[f]	22.93 ± 0.08[fg]	1.067 ± 0.040[e]	325.85 ± 2.10[c]	10.06 ± 0.07[ab]
Dexiang 4103		11.30 ± 0.12[f]	0.432 ± 0.010[de]	318.50 ± 7.52[a]	7.98 ± 0.19[e]	22.40 ± 0.04[h]	0.982 ± 0.041[e]	326.00 ± 3.75[c]	7.93 ± 0.34[f]
Gangyou 527		11.43 ± 0.25[f]	0.502 ± 0.024[b]	312.30 ± 3.40[ab]	8.45 ± 0.30[bcd]	22.70 ± 0.17[gh]	1.183 ± 0.035[d]	326.05 ± 4.44[c]	9.77 ± 0.19[bc]
Chuanxiang 9838		12.86 ± 0.00[d]	0.459 ± 0.022[cd]	306.21 ± 3.88[bc]	8.32 ± 0.22[de]	23.93 ± 0.06[e]	1.039 ± 0.054[e]	323.10 ± 4.24[cd]	9.14 ± 0.14[de]
Mean		12.16	0.457	308.16	8.33	23.01	1.060	325.23	9.44
L		1517.02**	12.16**	50.87**	26.43**	1412.60**	78.71**	4.39	35.55**
V		68.77**	12.34**	15.90**	23.55**	42.64**	11.62**	4.87**	74.73**
L × V		65.82**	8.82**	4.75**	14.81**	72.93**	29.67**	7.78**	26.46**

L, light regime and V, variety. Values are means ± SD of three independent replications (n=3). Different letters within the same column indicate statistically significant differences between treatments, according to Fisher's protected least significance differences test at the 5% level. ** $P<0.01$.

Table 3. Correlation coefficients between carbon isotope discrimation (Δ) of rice organs and photosynthetic characteristics in plants subjected to shading treatment in 2010–2011.

Year	2010			2011			
Treatment	ΔL_g	ΔS_g	ΔG_m	ΔL_g	ΔS_g	ΔS_m	ΔF_m
Full sunlight							
P_n	-0.032	-0.210	-0.257	0.488	0.342	-0.615*	-0.684**
Cond	-0.122	0.371	0.432	0.751**	0.673**	-0.823***	-0.186
C_i	0.208	0.178	0.682**	0.592*	0.518*	-0.684**	-0.089
Tr	0.526*	-0.622*	0.555*	-0.485	-0.482	0.490	-0.056
Shading							
P_n	-0.646**	-0.610*	-0.798**	0.078	-0.338	0.636*	-0.115
Cond	0.027	-0.319	0.115	-0.299	-0.598*	0.400	0.226
C_i	-0.088	-0.096	0.375	-0.037	-0.203	-0.119	0.098
Tr	-0.442	-0.530*	-0.355	-0.143	0.028	-0.091	-0.235
Across							
P_n	-0.303	-0.229	-0.759***	0.345	-0.319	-0.542**	-0.659***
Cond	-0.023	-0.059	0.000	0.452*	0.086	-0.596***	-0.344
C_i	0.008	-0.059	0.735***	0.383*	0.200	-0.492**	-0.150
Tr	-0.224	-0.462*	-0.168	-0.310	-0.056	0.253	0.021

P_n, photosynthetic rate; Cond, conductance to H_2O; C_i, intercellular CO_2 concentration; Tr, transpiration rate; ΔL_g, Δ of leaves at the grain-filling stage; ΔS_g, Δ of stems plus sheaths at the grain-filling stage; ΔG_m, Δ of rice grains at maturity; ΔS_m, Δ of stems plus sheaths at maturity; ΔF_m, Δ of rice flour at maturity. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Relationship between chlorophyll fluorescence characteristics and carbon isotope discrimination

There were substantial relationships between chlorophyll fluorescence characteristics and carbon isotope discrimination across or within light regimes (Table 4). Maximum efficiency of PSII photochemistry under dark adaption (F_v/F_m) was significantly positively associated with ΔS_g ($P<0.001$) across light regimes and with ΔL_g ($P<0.01$) and ΔS_g ($P<0.05$) under control conditions. However, highly ($P<0.05$) negative relationships were found between F_v/F_m and ΔL_g under shading treatment and ΔS_m and ΔF_m under control conditions. Quantum yield of PSII (Φ_{PSII}) was significantly ($P<0.05$ or $P<0.01$) negatively related to ΔS_m across light regimes and under shading treatment. Photochemical quenching (qP) was markedly negatively associated with ΔS_m and with ΔF_m across light regimes and under control conditions. In contrast, the correlation between qP and ΔL_g was significantly ($P<0.05$) positive under control conditions. In addition, non-photochemical quenching (NPQ) was highly negatively associated with ΔS_m and ΔF_m across light regimes and with ΔS_m under shading and ΔF_m under control conditions.

Table 4. Correlation coefficients between carbon isotope discrimination (Δ) of rice organs and chlorophyll fluorescence characteristics of plants subjected to shading treatment in 2011.

Treatment	ΔL_g	ΔS_g	ΔS_m	ΔF_m
Full sunlight				
F_v/F_m	0.665**	0.518*	-0.741**	-0.584*
Φ_{PSII}	0.284	0.226	-0.473	-0.130
qP	0.533*	0.435	-0.793***	-0.585*
NPQ	0.398	0.330	-0.441	-0.617*
Shading				
F_v/F_m	-0.605*	0.209	-0.153	0.228
Φ_{PSII}	-0.067	0.304	-0.578*	-0.258
qP	-0.006	0.321	-0.607*	-0.305
NPQ	-0.195	0.474	-0.650**	-0.187
Across				
F_v/F_m	-0.178	0.573***	0.288	0.344
Φ_{PSII}	0.109	0.102	-0.547**	-0.274
qP	0.240	0.022	-0.734***	-0.530**
NPQ	0.221	0.252	-0.447*	-0.471**

F_v/F_m, maximum efficiency of PSII photochemistry under dark adaption; Φ_{PSII}, quantum yield of PSII; qP, photochemical quenching; NPQ, non-photochemical quenching; ΔL_g, Δ of leaves at the grain-filling stage; ΔS_g, Δ of stems plus sheaths at the grain-filling stage; ΔS_m, Δ of stems plus sheaths at maturity; ΔF_m, Δ of rice flour at maturity. * P<0.05, ** P<0.01, *** P<0.001.

Relationship between grain yield and carbon isotope discrimination

Across light regimes, grain yield was significantly negatively (P<0.001) correlated with ΔG_m in 2010 and closely associated with ΔS_g, ΔS_m and ΔF_m in 2011 (Table 5). Furthermore, a markedly (P<0.001) positive correlation between grain yield and ΔF_m under control conditions was observed in 2011. Grain filling was highly (P<0.01) negatively related to ΔG_m in 2010 and also with ΔS_m and ΔF_m across light regimes in 2011. Grain weight was clearly negatively associated with ΔL_g across light regimes and under shading treatment in 2010 and with ΔS_m and ΔF_m across light regimes and under control conditions in 2011. Moreover, the relationships between grain weight and ΔS_g were significantly (P<0.05) positive under control and shading treatments in 2010 and 2011, respectively.

Table 5. Correlation coefficients between carbon isotope discrimination (Δ) of rice organs and grain yield, grain filling and grain weight in plants subjected to shading treatment in 2010–2011.

Year	2010			2011			
Treatment	ΔL_g	ΔS_g	ΔG_m	ΔL_g	ΔS_g	ΔS_m	ΔF_m
Full sunlight							
Grain yield	-0.087	-0.169	-0.468	-0.215	-0.055	0.449	0.826***
Grain filling	0.049	0.113	-0.401	0.117	-0.004	-0.214	-0.255
Grain weight	-0.450	0.566*	-0.128	0.670**	0.483	-0.784***	-0.623*
Shading							
Grain yield	0.097	0.222	0.009	0.332	0.300	-0.245	-0.234
Grain filling	0.093	0.058	-0.323	-0.307	0.592*	-0.558*	0.103
Grain weight	-0.646**	-0.290	-0.080	0.211	0.563*	-0.343	-0.273
Across							
Grain yield	0.000	0.099	-0.743***	0.207	-0.437*	-0.522**	-0.404*
Grain filling	0.023	0.094	-0.739***	0.141	-0.323	-0.670***	-0.496**
Grain weight	-0.534**	-0.055	-0.194	0.444*	-0.047	-0.729***	-0.649***

ΔL_g, Δ of leaves at the grain-filling stage; ΔS_g, Δ of stems plus sheaths at the grain-filling stage; ΔG_m, Δ of rice grain at maturity; ΔS_m, Δ of stems plus sheaths at maturity; ΔF_m, Δ of rice flour at maturity. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Discussion

Difference in Δ among rice organs

Carbon isotope discrimination varied among plant organs. In this study, the ΔG_m or ΔF_m was obviously lower than ΔL_g, ΔS_g and ΔS_m (Table 1), which is consistent with the earlier findings of Zhao et al. (2004), who reported a lower Δ in rice grains compared to leaves and stems during different developmental stages. However, stems plus sheaths had higher Δ than leaves, whereas an increasing tendency was observed in ΔS concomitant with plant growth. These observations might be explained by reference to Brugnoli and Farquhar (2000), who pointed out that fractionation during the export and transport of carbohydrate from photosynthetic to storage organs and differences in the chemical composition of each organ, might be why Δ varies in different plant organs. Furthermore, differences in the Δ values of plant organs varied markedly among varieties (Table 1), which is in accordance with the results of Moghaddam et al. (2013) and Zhao et al. (2004).

Effect of shading treatment on Δ

Under natural growing conditions, plants are subjected to various factors that can cause Δ to vary. Shaheen and Hood-Nowotny (2005) reported that soil salinity considerably decreased the Δ of wheat leaves. Drought stress also markedly reduced the

Δ of leaves and grains (Farquhar and Richards, 1984; Akhter et al., 2003; Kondo et al., 2004; Misra et al., 2010). Furthermore, plant management, such as tillage and use of allelochemicals, has a clear influence on the Δ of plant organs (Iqbal et al., 2005; Hussain et al., 2011). In the present study, shading treatment markedly decreased the ΔL_g in 2011, partly due to a decrease in C_i (Table 2); this is because the Δ of C_3 plant leaves is associated with C_i/C_a, the ratio of leaf intercellular CO_2 concentration (C_i) to atmospheric CO_2 concentration (C_a) (Farquhar and Richards, 1984; Shaheen and Hood-Nowotny, 2005). On the other hand, shading treatment significantly ($P<0.05$) increased ΔG_m in 2010 and ΔS_g, ΔS_m and ΔF_m in 2011 (Table 1). This might be a consequence of the perturbation of photosynthetic and chlorophyll fluorescence characteristics caused by shading treatment (Mu et al., 2010; Wang et al., 2015), which results in differences in carbohydrate accumulation, distribution and transportation. Additionally, unlike water regimes (Zhao et al., 2004), the response of Δ to shading treatment was significantly ($P<0.01$) related to rice varieties. For example, in both years of the study, shading treatment increased the ΔL_g and ΔS_g of Gangyou 188 and Gangyou 527, but reduced these values in Chuanxiang 9838. These results indicate the potential use of Δ for selecting highly shade-tolerant rice varieties under shading treatment.

Interrelationships between Δ, grain yield and photosynthetic and chlorophyll fluorescence characteristics

The correlations between Δ and grain yield and plant growth characteristics have been investigated by a numbers of researchers. Merah et al. (2000 and 2001) have reported that the Δ of grains and leaves were significantly ($P<0.001$) positively correlated with grain yield under different environments. Akhter and Monneveux (2012) suggested that the Δ of plant organs (leaf or grain) was positively correlated with grain yield in a wide range of crops. However, the association between grain yield and Δ was highly dependent on the environmental conditions (Kondo et al., 2004; Misra et al., 2010). Further, the correlation between the Δ of leaves and harvest index was closely related to plant varieties and growth conditions (Shaheen and Hood-Nowotny, 2005). This is in agreement with our study, in which we found that the grain yield of rice was significantly ($P<0.001$) positively associated with ΔF_m under control conditions, but substantially negatively related to ΔG_m, ΔS_m and ΔF_m across light regimes (Table 5).

Moreover, there were clear relationships between Δ and photosynthetic and chlorophyll fluorescence characteristics (Tables 3 and 4), which are closely related to grain yield (Wang et al., 2015). Iqbal et al. (2005) reported that the nitrogen concentration of biomass, which is closely related to the photosynthetic and chlorophyll fluorescence characteristics of plants, was positively associated with the Δ of plant biomass. In this study, similar relationships between ΔS_m and chlorophyll fluorescence characteristics were observed under control and shading treatments (Table 4). However, the correlation between ΔS_g and Cond was positive under control conditions, but negative under shading treatment. In contrast, ΔS_m was negatively associated with P_n under control conditions but significantly ($P<0.05$) positively associated with P_n under shading treatment (Table 3). In agreement with the report of Moghaddam et al. (2013), the results of our study suggest that the relationship of plant growth characteristics and consequently that of grain yield with the Δ responses of varieties, varied depending on plant organ (leaf, stem plus sheath, grain and flour), growth stage and light regime (Tables 3, 4 and 5).

Potential use of Δ for selecting highly shade-tolerant varieties

Carbon isotope discrimination can provide an integrated insight into plant performance over a wide range of conditions (Kennedy et al., 2006; Cernusak et al., 2013). A high Δ indicates a high C_i/C_a and either high stomatal conductance or low assimilatory capacity (Ngugi et al., 1996) and the measurement of Δ has become a useful tool for providing additional insight into the photosynthetic metabolism of C_3 species, as well as the environmental influences on this process (Farquhar et al., 1989). Under shading treatment, the P_n was closely related to the Δ of plant organs, whereas ΔS_m was markedly associated with chlorophyll fluorescence characteristics (Tables 3 and 4). Furthermore, the carbon isotope discrimination technique has been proposed as an indirect means of selection for grain yield and resource utilization (such as water-use efficiency) improvement in cereals (Merah et al., 2000; Misra et al., 2010; Akhter and Monneveux, 2012), as well as a potential physiological index for identifying shade-tolerant species (Kennedy et al., 2006). In this study, both grain filling and grain weight were significantly (positive or negative, P<0.05) associated with the Δ of some plant organs under shading treatment (Table 5), indicating the potential use of Δ for selecting shade-tolerant rice varieties. For example, the selected highly shade-tolerant variety IIyou 498 had lower ΔL_g, ΔG_m and ΔS_m (Table 1) and higher P_n, F_v/F_m, Φ_{PSII} and qP, resulting in less crop failure by maintaining relatively higher grain filling and grain weight (Wang et al., 2015).

Conclusion

Shading treatment had substantial effects on the carbon isotope discrimination of rice. However, the effectiveness of shading treatment on Δ was dependent on rice variety, plant organ and growth stage. Shading treatment markedly increased ΔG_m, ΔS_m and ΔF_m, but reduced ΔL_g. There were close associations between Δ and grain filling, grain weight and also the photosynthetic and chlorophyll fluorescence characteristics of rice leaves, which are closely with grain yield. Under shading treatment, P_n was significantly negatively related to ΔS_g and ΔG_m, whereas ΔS_m was markedly negatively associated with Φ_{PSII}, qP and NPQ. Furthermore, grain filling and grain weight were positively correlated with ΔS_g but negatively correlated with ΔS_m and ΔL_g, respectively. Taken together, the measurement of Δ could be used as a potential method for selecting highly shade-tolerant varieties of rice. Moreover, the lower values of ΔL_g, ΔS_m and ΔG_m indicate better light-harvesting and light-use capability of rice leaves, as well as higher grain filling and grain weight of rice. However, the use of Δ was limited by rice organ and growth stage.

Acknowledgements

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