An in situ approach for characterizing photosynthetic gas exchange of rice panicle

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Methodology

Keywords: rice panicle, reproductive organs, in situ, photosynthetic characteristics phenotyping, grain setting rate, P-chamber

Posted Date: January 15th, 2020

DOI: <https://doi.org/10.21203/rs.2.20971/v1>

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Version of Record: A version of this preprint was published on July 6th, 2020. See the published version at <https://doi.org/10.1186/s13007-020-00633-1>.

Abstract

Background: Photosynthesis of reproductive organs in C₃ cereals is generally regarded as important to crop yield. Whereas, photosynthetic characteristics of reproductive organs are much less understood as compared to leaf photosynthesis, mainly due to methodological limitations. To date, many indirect methods were developed to study photosynthesis of reproductive organs and its contribution to grain yield, such as shading, application of herbicides or photosynthetic measurement of excised organs or tissues, which might be intrusive and cause biases. Thus, a robust and in situ approach needs to be developed.

Results: Here we report the development of a custom-built panicle chamber (P-chamber), which can be connected to a standard infrared gas analyzer to study photosynthetic/respiratory rate of a rice panicle. With this P-chamber, we measured photosynthetic characteristics of panicles for seven high-yielding elite japonica, japonica-indica hybrid and indica rice cultivars. Results show that, 1) rice panicle is photosynthetically active during grain filling, and there are substantial inter-cultivar variations in panicle photosynthetic and respiratory rates, both for a whole panicle and on a single spikelet basis; 2) among the seven testing cultivars, light saturated whole-panicle gross photosynthetic rates at early grain filling stage are 17 – 54 nmol s⁻¹, which are estimated to be 20-38% of the corresponding flag leaves; 3) rice panicle photosynthesis has higher apparent CO₂ compensation point, light compensation point and apparent CO₂ saturation point, as compared to that of a typical leaf; 4) there is a strong and significant positive correlation between gross photosynthetic rate at early grain filling stage on a single spikelet basis and grain setting rate at harvest (Pearson correlation coefficient $r = 0.93$, p -value < 0.0001).

Conclusions: Overall, the P-Chamber can be used as a tool to study in situ photosynthetic characteristics of irregular non-foliar plant organs, which is a relatively less explored area in current cereal breeding community.

Introduction

In cereal crops such as rice and wheat, there are two major sources of assimilates used for grain filling, i.e., the pre-anthesis assimilates stored in the stem and the post-anthesis photosynthesis. Usually, the post-anthesis photosynthesis contributes greater to grain yield than the pre-anthesis stored assimilates, especially under non-stress conditions [1–4]. In cereals such as rice and wheat, leaves, especially flag leaves located at the upmost of stems, have been believed as the dominant source of post-anthesis photosynthesis [5, 6]. Nevertheless, reproductive organs, including glumes, caryopsis pericarp, and awns, are green before mature and also photosynthetically active [7, 8], suggesting that they may be another major source of photosynthate for grain filling as well. In fact, reproductive organs have several potential advantages over leaves in terms of photosynthate production: 1) they generally locate at the upper canopy, where light levels are usually higher than those under lower canopy; 2) they are physically closer to the developing grains than the leaves, which shorten the path of assimilates transport to developing grains [9]; 3) they have large surface area, e.g. the ear surface areas were 1.1–5.5 folds greater than that of the corresponding flag leaves in wheat and barley [6]; 4) reproductive organs tend to senesce later than leaves, especially under adverse conditions such as drought [10, 11]. Due to these reasons, photosynthesis of reproductive organs gained more attraction in recent years (see review in [12, 13]).

In wheat and barley, ear photosynthesis is a major source of photosynthate for grain filling; a large photosynthetic area and high photosynthetic activity from both the green glumes and awns are major factors underlying higher ear photosynthesis [6, 14, 15]. Using an ear-shading and/or stem-shading method, ear photosynthesis was estimated to contribute 10–65% of the grain yield in different wheat lines and under different growth conditions [14–18]. By analyzing the carbon isotope composition in different organs, it was estimated that ear photosynthesis contributed 47–100% of the grain yield in six high-yielding bread wheat lines under well-managed conditions [19, 20]. In a retrospective

study, by using 15 winter wheat cultivars released from 1980 to 2012 in North China Plain, it was reported that the contribution of ear photosynthesis to grain yield doubled (from around 30% to around 60%) during breeding [16]. Although the exact proportion of ear photosynthesis to total grain filling varies, breeding cultivars with higher ear photosynthesis was recognized as a new mechanism to improve wheat yield potential [21].

In contrast to the significant importance of ear photosynthesis in wheat and barley, rice panicle has been traditionally regarded mainly as a sink organ with negligible photosynthetic contribution to grain yield [22]. For example, Tsuno et al. (1975) estimated that net panicle photosynthesis is about 5% of that of an active photosynthesizing rice leaf [23]; Takeda and Maruta (1956) estimated that panicle photosynthesis contributes 8% of the rice grain yield [24]. Reflecting the notion of rice ear photosynthesis being of little importance to grain filling, some researchers recommended lowering spike position in a canopy as much as possible to increase canopy photosynthesis by minimizing shading leaves by panicles [25].

Is panicle photosynthesis in rice really of little importance for grain filling? Many evidences show this is not the case. Imaizumi et al. (1990) showed that whole-panicle gross amount of photosynthetically assimilated CO₂ was 30% of that in a flag leaf, and spikelets had a similar photosynthetic capability to a flag leaf on the basis of Chl [8]. It has been shown that rice panicle contains higher amount of chloroplast NAD(P)H dehydrogenase-like complex, and maintains a similar or higher quantum yield of PSII than that of the flag leaf from heading to harvest [26]. There are also evidences showing an important contribution of rice panicle photosynthesis to grain filling under moderate stress conditions. For example, it was found that under moderate wetting and drying irrigation after anthesis, the expression of proteins that function in photosynthesis and carbohydrate metabolism were upregulated in inferior spikelets, together with a simultaneous increase of grain filling rate and grain weight of the inferior spikelets [27].

Although photosynthesis of reproductive organs could be important for grain filling, so far our knowledge on photosynthesis of reproductive organs is rather limited, largely due to methodological limitations in dealing with the complex three-dimensional geometry of reproductive organs [12, 20, 21]. Many indirect methods have been developed to study of reproductive organs [21], e.g. through ear (awn) removal/shading or application of herbicides [15, 25, 28], isotope tracing [19], gas exchange measurement of excised spikelets [6, 8] and chlorophyll fluorescence measurement for plants cultured in pots [26]. Among these methods, the shading treatments or applications of herbicides may affect function of organs or trigger compensation effects of unaffected organs [18, 20, 29–33]. The stable isotope discrimination method compared instant carbon isotope in water-soluble fraction in different tissues at mid-grain filling stage and carbon isotope in mature grains, the accuracy of which might be affected by the dynamic environment (e.g. temperature and air humidity) and physiological processes other than photosynthesis (e.g. assimilates transport and ear night respiration) during the whole grain filling season [12, 19, 20]. Gas exchange measurement on excised ears or spikelets is an intrusive method; furthermore, this measurement might cause bias due to an alteration of ear natural architecture and posture [25].

Thus, the main objectives of this study are: 1) to develop a direct and non-intrusive method for gas exchange measurement of rice panicles; 2) to explore panicle photosynthetic characteristics and the inter-cultivar variation of photosynthetic gas exchange parameters; 3) and to study the relationship between panicle photosynthetic gas exchange parameters and grain yield related agronomic traits.

Materials And Methods

Plant materials and experimental design

Rice plants were grown in a paddy field at Songjiang breeding station of the Institutes of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China (30°56' 44" N, 121°8' 1" E) in 2015 and 2016. Seeds were sown on seedbeds after germination on 1 June 2015 and 5 June 2016, and seedlings were transplanted to the field on 26 June 2015 and 29 June 2016. In both years, one plant was transplanted into each hill with a spacing of 20 cm between hills and 20 cm between rows. The cultivars were planted in plots. Plots were arranged in blocks with five replicates, each containing 56 (7 × 8) plants. Basal fertilizer was applied at a rate of 120 kg N ha⁻¹, 65 kg P ha⁻¹ and 65 kg K ha⁻¹. Additional N fertilizer was top-dressed at a rate of 80 kg N ha⁻¹ 3 weeks after transplanting. Weeds, pests and diseases were controlled following common agronomic practice in the region. In 2015, five rice cultivars were grown for experiments: two recently bred *indica* super high yield hybrid cultivars Y-Liang-You 900 (YLY900; <http://www.ricedata.cn/variety/varis/614537.htm>) and Chao-You 1000 (CY1000; <http://www.ricedata.cn/variety/varis/616642.htm>), one widely grown traditional *indica* hybrid cultivar Shan-You 63 (SY63; <http://www.ricedata.cn/variety/varis/601174.htm>), one *indica* inbred cultivar Yang-Dao 6 (9311; <http://www.ricedata.cn/variety/varis/600611.htm>) and one elite *japonica* inbred cultivar Xiu-Shui 134 (XS134; <http://www.ricedata.cn/variety/varis/603176.htm>). In 2016, in spite of the above mentioned 5 cultivars, an additional two *japonica-indica* hybrid cultivars Yong-You 538 (YY538; <http://www.ricedata.cn/variety/varis/613563.htm>) and Yong-You 17 (YY17; <http://www.ricedata.cn/variety/varis/612347.htm>) were grown for experiments. In 2015, the heading date and harvest date of main stems for rice cultivars YLY900, CY1000, SY63, 9311 and XS134 were 2 Sep., 2 Sep., 27 Aug., 27. Aug., 6 Sep. and 25 Oct., 25 Oct., 25 Oct., 25 Oct., 5 Nov., respectively. In 2016, the heading date and harvest date of main stems for rice cultivars YLY900, CY1000, SY63, 9311, XS134, YY538 and YY17 were 4 Sep., 4 Sep., 29 Aug., 29. Aug., 7 Sep., 7 Sep., 15 Sep. and 28 Oct., 28 Oct., 28 Oct., 28 Oct., 8 Nov. , 8 Nov. , 8 Nov., respectively.

Construction of the custom-designed panicle chamber

To enable direct and non-intrusive measurement of panicle photosynthesis, we developed a custom-designed panicle photosynthesis measurement chamber (P-chamber), which can be used together with an infrared gas analyzer to measure photosynthetic and respiratory rates of a whole rice panicle. The chamber body is made of aluminum, with cooling fins and cooling fans on both sides (Fig. 1A). The chamber body has a dimension of 30 × 5 × 5 cm. The air inside the chamber was mixed with two air-mixing fans inside the chamber (F2008ES-05WAV, SHICOH IC FAN, Japan), light source and a light intensity control system (Fig. 1A, B). The inner space is uniformly illuminated with 192 light-emitting diodes (LEDs; CREE XML T6, Guangdong Benbon Electrical Co., Ltd) on an 30 × 5 cm Aluminum substrate (Dongxing electronic technology Co., Ltd) for which the photon flux densities can be precisely controlled to span a photon flux density (PFD) from 0 to 2000 μmol m⁻² s⁻¹ (Fig. 1B). The PFDs in the P-chamber under different input voltages were calibrated with an irradiance sensor (LightScout Light Sensor Reader, LightScout, Spectrum Technologies, Inc. USA) to build the relationship between input voltage and PFD. During measurement, the P-chamber is connected to a standard Infra-red gas analyzer, and the rice panicle is held upright and fully enclosed in the chamber (Fig. S1). Flow rate and CO₂ concentration of influx air are controlled by LI-6400, and the data are recorded and stored in LI-6400.

Panicle photosynthetic gas exchange parameters measurement

Twenty panicles were randomly selected and labeled at heading for each rice cultivar, which were used to measure photosynthetic gas exchange parameters and yield related agronomic traits. Three and five panicles among these twenty were used to measure whole-panicle net photosynthetic rate and dark respiratory rate in 2015 and 2016, respectively. The photon flux density used for each measurement of net photosynthetic rate was 2000 μmol m⁻² s⁻¹; the

CO₂ concentration was set to 400 $\mu\text{mol mol}^{-1}$, the flow rate was set to 700 $\mu\text{mol s}^{-1}$; then dark respiratory rate was measured by setting the PFD to 0. Each time when the readings were stabilized, data were logged after matching the two infrared gas analyzers (IRGAs).

The rice cultivar YY17 was used for photosynthetic light and CO₂ response curves measurements in 2016 at early grain filling stage. For photosynthetic light response curve measurement, the CO₂ concentration was set to 400 $\mu\text{mol mol}^{-1}$, the flow rate was set to 700 $\mu\text{mol s}^{-1}$; and the light levels were manually changed to 2000, 1500, 1000, 700, 500, 300, 200, 100, 50, 25 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during measurement, respectively. For photosynthetic CO₂ response curve measurement, the light intensity was set to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the flow rate was set to 700 $\mu\text{mol s}^{-1}$; and the CO₂ concentrations were manually changed to 400, 300, 150, 100, 50, 400, 600, 900, 1200, 1500, 1800 $\mu\text{mol mol}^{-1}$ during measurement, respectively.

Agronomic traits measurement

Five of the twenty labeled panicles were sampled and stored in envelopes individually for each cultivar at harvest. The panicle length was measured, then the panicles were put in an oven for sample drying. The temperature of the oven was maintained at 110 °C for 1 hour, and then switched to 70 °C and maintained for 3 days until samples were completely dried. Dry weight of each panicle was measured with an electronic balance AL104 (JB / T, METTLER TOLEDO, USA). The panicles were then threshed to determine the total spikelet number and filled grain number. The weight of all filled grains were measured to determine the 1000-grain weight.

Data Analysis

Statistical analyses were carried out using R-project (version 3.6.2). One-way analysis of variance (ANOVA) and a Fisher's least significant difference (LSD) post-hoc test were used to compare data at a level of 5%. The statistically significant *p*-values were adjusted using Bonferroni's method. The Pearson correlation coefficients between panicle photosynthetic gas exchange parameters and grain yield related agronomic traits were calculated using the R package (Corrplot; version 0.84).

Results

Performance of the P-chamber

The P-chamber is connected to a standard gas analyzer (Li-6400, Li-Cor Inc., Lincoln, Nebraska, USA) to measure gas exchange rate of a rice panicle (Fig. S1). The panicle is illuminated with LED light source (Fig. 1A, B). The LED light source emits pure white light (light wavelength information is shown in Fig. 1C). The light intensity was in a range of $\pm 10\%$ of set values on 87% of the total 30 $\times$ 5 cm light field (Fig. S2). At a room temperature of 25 °C with a flow rate of 700 $\mu\text{mol s}^{-1}$, temperature rise in the chamber at a light intensity of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was 4.3 ± 0.7 °C.

To test the leakage of the P-chamber, we recorded $\Delta[\text{CO}_2]$ (i.e., the difference between CO_2 concentration in the P-Chamber and the set reference CO_2 concentration in the reference pipe) at different time after closure of the P-chamber. Results showed that with an initial $\Delta[\text{CO}_2]$ value of around $500 \mu\text{mol mol}^{-1}$, the $\Delta[\text{CO}_2]$ values were stabilized within 8 min under different flow rates of 500 and $700 \mu\text{mol s}^{-1}$ and different set CO_2 concentrations of 0 and $400 \mu\text{mol mol}^{-1}$ (Fig. 1D). The final $\Delta[\text{CO}_2]$ values were $\leq 1.6 \mu\text{mol mol}^{-1}$ and $\leq 1.3 \mu\text{mol mol}^{-1}$ at a flow rate of 500 and $700 \mu\text{mol s}^{-1}$, respectively (Fig. 1D). During a typical measurement of panicle photosynthetic rate and respiratory rate, the light source was turned on before the rice panicle was enclosed, and the photosynthetic rate was recorded after stabilization; then the light source was turned off, and the respiratory rate was recorded after stabilization (Fig. 1E). The entire measurement of photosynthetic rate and respiratory rate for one rice panicle usually took 10 - 20 min after the panicle was enclosed in the P-chamber, depended on size and photosynthetic activity of the panicle.

Characterization of rice panicle photosynthesis

We first used the P-chamber to measure the *in situ* whole-panicle photosynthetic gas exchange parameters 5 days after heading for different rice cultivars when the panicles were still erect or semi-erect, i.e., whole-panicle light saturated net photosynthetic rate (total A_{net}) and dark respiratory rate (total R_d). Whole-panicle gross photosynthetic rate (total A_{gross}) was calculated as a sum of total A_{net} and total R_d . In addition, average A_{gross} , A_{net} and R_d of a single spikelet on a panicle were calculated by dividing total A_{gross} , A_{net} and R_d by the spikelet number of a panicle.

Results showed that, firstly, there are substantial inter-cultivar variations in photosynthetic gas exchange parameters among rice cultivars, for both values of a whole panicle and values normalized by spikelet number. For example, the average A_{net} of a spikelet and the total A_{net} of a whole panicle were $0.012 - 0.051 \text{ nmol s}^{-1}$ and $2.7 - 12.0 \text{ nmol s}^{-1}$ in 2015, and were $-0.014 - 0.075 \text{ nmol s}^{-1}$ and $-3.3 - 15.0 \text{ nmol s}^{-1}$ in 2016, respectively (Table 1). Secondly, *indica* type rice cultivars (YLY900, CY1000, SY63 and 9311) tended to have higher A_{gross} , A_{net} and R_d than *japonica* and *japonica-indica* hybrid type rice cultivars (XS134, YY538 and YY17), both on whole panicle basis and on single spikelet basis. Moreover, light saturated A_{gross} of a panicle could be as high as 17.1 to 53.6 nmol s^{-1} (Table 1).

We further measured the dynamic changes of panicle total A_{gross} and R_d for seven rice cultivars during grain filling in 2016 (Fig. 2). Results showed that, firstly, panicle total A_{gross} and R_d were highest at early grain filling stage, then slowly decreased with time. Notably, both A_{gross} and R_d could still be detected even 40 days after heading, i.e., panicle photosynthesis and respiration were active throughout the grain-filling season. It is worth mentioning here that the panicle total A_{gross} should be interpreted as the maximal photosynthetic potential, as the panicle was held upright during measurement, which was not the natural state after early grain filling stage for some cultivars.

Finally, we measured the photosynthetic light and CO_2 response patterns of a panicle using rice cultivar YY17. Although the shapes of both response curves are similar to that of a leaf, there are several differences. Firstly, the apparent CO_2 and light compensation points of a panicle were about $180 \mu\text{mol mol}^{-1}$ and $390 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig. 3), much higher than those of a leaf, which were usually $<100 \mu\text{mol mol}^{-1}$ and $<50 \mu\text{mol m}^{-2} \text{ s}^{-1}$, respectively. Secondly, the panicle

photosynthetic rate was still not saturated at very high air CO₂ concentration (1800 μmol mol⁻¹; Fig. 3A), which also differed from that of a typical leaf.

Correlation between panicle photosynthetic gas exchange parameters and grain yield related agronomic traits

We further studied the relationship between panicle photosynthetic gas-exchange parameters measured 5 days after heading (Table 1) and six grain yield related agronomic traits collected at harvest across two years, i.e., spikelet number per panicle, panicle length, spikelet density (defined as spikelet number per centimeter of panicle length), 1000-grain weight, grain setting rate and panicle weight (Table 2). Firstly, there are positive correlations between different panicle photosynthetic gas-exchange parameters (Fig. 4). For example, significant positive correlations were found between A_{gross} and A_{net} of a whole panicle (Pearson correlation coefficient $r = 0.87$, p -value < 0.001), between A_{gross} and R_d of a whole panicle ($r = 0.94$, p -value < 0.0001), between A_{gross} and A_{net} per spikelet ($r = 0.93$, p -value < 0.0001), and between A_{gross} and R_d per spikelet ($r = 0.97$, p -value < 0.0001 ; Fig. 4). In contrast, both positive and negative correlations were found between grain yield related agronomic traits, e.g. significant negative correlations were found between 1000-grain weight and spikelet number per panicle, but significant positive correlations were found between 1000-grain weight and grain setting rate (Fig. 4). Notably, strong positive correlations were found between A_{gross} , A_{net} , R_d per spikelet and grain setting rate, 1000-grain weight. In particular, the Pearson correlation coefficient r exceeds 0.9 (p -value < 0.0001) between A_{gross} per spikelet and grain setting rate (Fig. 4). Concurrently, results showed that A_{gross} , A_{net} and R_d of a whole panicle had a moderate positive correlation with panicle dry weight at harvest ($r = 0.39 \sim 0.55$); whereas A_{gross} , A_{net} and R_d per spikelet were not correlated with panicle dry weight at harvest ($r = -0.18 \sim 0.12$; Fig. 4). In addition, all the six panicle photosynthetic gas exchange parameters were found to be positively correlated with panicle length ($r = 0.6 \sim 0.85$), whereas they were negatively correlated with spikelet density ($r = -0.82 \sim -0.28$; Fig. 4). No correlations were found between A_{gross} , A_{net} , R_d of a whole panicle and spikelet number per panicle ($r = -0.2 \sim 0.1$; Fig. 4).

Discussion

Reproductive organs of cereal crops have been shown to be photosynthetically active, and photosynthesis in ear of wheat and barley has attracted great research interest [12, 13]. Current high-yield rice cultivars usually have much bigger ears with numerous spikelets compared with wheat or barley. For example, the newly bred rice cultivar YLY900, which has reached record-high yield of 15 tons ha⁻¹ [34], and another even higher yielding rice cultivar CY1000, have an extra-large panicle size of exceeding 350 and 400 spikelets per panicle, respectively (Table 2). As a result, the green area of a rice panicle can be large. Furthermore, considering that the panicles locate at the upper part of a canopy, they can intercept a considerably amount of solar radiation. All these suggest a great role of panicle photosynthesis in rice grain filling. However, compared to leaf photosynthesis, panicle photosynthesis is much less studied, largely due to the methodological limitations [12, 20, 21]. Therefore, new tools and methods are needed to enable better characterization of rice panicle photosynthesis and provide an accurate evaluation of the impact of panicle photosynthesis on rice grain yield.

Here we reported the development of a new chamber (P-chamber), which can be connected to a standard infrared gas analyzer to enable direct measurement of gas exchange parameters. Compared to previously reported chambers for rice or wheat ear gas exchange measurement [8, 15, 20, 35], the P-chamber has several new features. First, it is made air-tight with very limited leakage under different CO₂ concentrations and flow rates, which enables accurate and fast measurement of gas exchange rate. Secondly, it has a large cuvette volume for gas exchange measurement of a whole

rice panicle in situ. The large cuvette volume enables measurement of panicles of irregular shape. We expect that it can also be used to measure inflorescence of wheat, rape, soybean and etc. or other plant organs such as stem and branches in situ. Moreover, the chamber is opaque and the light intensity inside is uniformly distributed and can be precisely controlled, which enables studying response of photosynthesis to light intensity.

Using the P-chamber, we studied characteristics of rice panicle photosynthesis. Firstly, we found that at early grain filling stage, light saturated A_{gross} of a whole panicle could be as high as 17.1 to 53.6 nmol s^{-1} . With an assumed typical light saturated photosynthetic rate of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ [36–38], the A_{gross} of whole flag blades for the seven rice cultivars were 81.5 to 184.5 nmol s^{-1} , which resulted in the estimated A_{gross} ratio between panicle and flag leaf to be 19.9–38.1% (Table S1). This result is comparable to a previous report, where A_{gross} of a panicle is 30% of that in a flag leaf [8]. Concurrently, though panicle photosynthetic activity gradually decreases during grain filling, it is still detectable even 40 days after heading (Fig. 2). Maintaining photosynthetic activity of a panicle till the end of rice grain filling has been reported earlier as well [26]. The contribution of panicle photosynthesis to rice grain yield cannot be precisely calculated in the current study. If we assumed a constant respiratory rate during the daytime, i.e., from 6:00 AM to 18:00 PM, the total respired CO_2 during the whole grain filling season for the seven cultivars were 39.7, 39.3, 26.2, 34.1, 20.7, 28.1, 30.0 mmol (an integration of the Tot. R_d in Fig. 2), which were equivalent to 1.2, 1.2, 0.8, 1.0, 0.6, 0.8, 0.9 g CH_2O , respectively. This accounts for 12.1–17.0% of the panicle weight at harvest (Table 2). Namely, by refixation of daytime respiratory CO_2 , panicle photosynthesis can contribute up to 12.1–17.0% of the final grain yield, which is much higher than the previous estimation of 8% [24].

Secondly, there are substantial inter-cultivar variations in panicle photosynthetic and respiratory rates, both on a whole panicle basis and on a spikelet basis, especially between indica and japonica or japonica-indica hybrid rice cultivars. Indica cultivars tend to have higher panicle gross photosynthetic rates per spikelet than japonica or japonica-indica hybrid cultivars (Table 1). One of the main factors contributing to this difference might be panicle morphology. For example, the high-yielding japonica rice usually has erect and densely-packed panicle [39], while the indica rice cultivars typically have drooping and loosely-packed panicles [40]. Indeed, a negative correlation between spikelet density and gross photosynthetic rate per spikelet was found in this study (Fig. 4). Inter-subspecies differences in photosynthetic apparatus and metabolism may also exist, which needs to be further studied.

Moreover, rice panicles show differences in photosynthetic response pattern to light intensities and CO_2 concentrations. Specifically, rice panicle has high photosynthetic light compensation point and high apparent CO_2 compensation point, due to a high respiratory rate; and has high CO_2 saturation point, which might be due to different stomatal conductance response pattern. Consistently, it was also observed that ear had higher CO_2 compensation point, and much higher CO_2 saturation point compared with that of flag leaf in wheat [35].

We further studied correlation between panicle photosynthetic gas exchange parameters and grain yield related agronomic traits. Interestingly, we found that panicle photosynthetic rate and respiratory rate were closely inter-correlated, both for a whole panicle and for single spikelet averages (Fig. 4). Moreover, we found spikelet gross photosynthetic rates at early grain filling stage were strongly and significantly correlated with grain setting rate ($r = 0.93$) and 1000-grain weight ($r = 0.84$; Fig. 4). These results indicate that activity of the spikelet as a source and as a sink may closely linked. It is likely that the activity of the spikelet as a photosynthetic source promotes the activity of the grain as a sink. In this aspect, a gene (GFR1) was recently reported to improve grain-filling rate by increasing RuBisCO initial activity in the Calvin cycle [41]. The detailed mechanism of this “source-sink” correlation is still not clear. Earlier studies on wheat offer a few possibilities. Panicle photosynthesis may supply oxygen to the hypoxic regions deep within the developing seed to increase its metabolic activity [42]; furthermore, the transpiration accompanying panicle photosynthesis not only supports the transfer of nutrients and signal molecules from xylem to spikelets, but also

regulates panicle temperature to avoid over-heating [43, 44]; in addition, panicle photosynthesis can generate assimilatory power, i.e., ATP and reduced ferredoxin, which are required to support glume nitrogen assimilation, a major source of nitrogen in wheat grain [45].

The method of directly measuring reproductive organ gas exchange rate has a range of potential applications. Firstly, it can help accurately simulate photosynthesis from non-foliar organs and evaluate their role in canopy photosynthesis based on three-dimensional canopy photosynthesis modeling [46, 47]. Secondly, as large natural variations of panicle photosynthetic and respiratory rates exist between cultivars, the genetic basis can be explored by measuring panicle gas exchange parameters in rice genetic populations. Thirdly, the diurnal and seasonal panicle respiratory rate change can be measured to trace grain filling dynamics, which is of great importance in rice yield formation [38, 41].

Conclusions

Here we report the design and application of a new device, P-chamber. The features of large cuvette volume, air tight, and programmable light intensity inside the P-chamber enable in situ characterization of photosynthetic gas exchange of a whole plant organ. With the P-chamber, for the first time, we characterized whole-panicle photosynthetic light and CO₂ response patterns, quantified variations of panicle photosynthesis between cultivars and identified correlations between panicle photosynthetic gas exchange parameters and agronomic traits in rice. Further application of the P-Chamber will facilitate study on photosynthetic characteristics and contribution of irregular photosynthetic organs, especially the non-foliar photosynthetic organs, e.g. inflorescence of cereals and other plant organs such as stem and branches.

Abbreviations

P-chamber, custom-built panicle photosynthesis chamber; C_a, ambient CO₂ concentration; PFD, photon flux density; Tot. A_{net}, net photosynthetic rate of a whole panicle (nmol s⁻¹); Tot. A_{gross}, gross photosynthetic rate of a whole panicle (nmol s⁻¹); Tot. R_p, respiratory rate of a whole panicle (nmol s⁻¹); A_{net}, net panicle photosynthetic rate on a spikelet basis (nmol s⁻¹); A_{gross}, gross panicle photosynthetic rate on a spikelet basis (nmol s⁻¹); R_d, panicle respiratory rate on a spikelet basis (nmol s⁻¹); SN, spikelet number per panicle; PL, panicle length (cm); SD, spikelet density (cm⁻¹); TGW, 1000-grain weight (g); GSR, grain setting rate; PW, panicle dry weight (g).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in the article and in Additional file 1.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This research was financially supported by the CAS strategic priority research program of the Chinese Academy of Sciences (XDB27020105), National Science Foundation of China (grant No. 31870214), National Natural Science Foundation of China (grant No. 31871704) and Hunan Provincial Natural Science Foundation of China (grant No. 2018JJ2286).

Authors' contributions

XGZ and TGC conceived the research plan; TGC and HLZ managed the experiments; QFS and CX provided suggestions and technical assistance for the P-chamber building; TGC analyzed the data; TGC and XGZ drafted the article; all authors discussed the results and contributed to the final manuscript.

Acknowledgements: The authors thank Xiaojuan Fan, Yi Xiao, Han Jing, Mingnan Qu and Faming Chen for assistance in field experiments and helpful comments and suggestions on an early draft of the manuscript.

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Tables

Table 1. Panicle photosynthetic gas exchange parameters of the rice cultivars measured 5 days after heading. Data presented are mean values with s.d. (n = 3 in year 2015; n = 5 in year 2016). Data followed by different letters are statistically different at *p*-value < 0.05.

Year	Cultivar	Tot. A_{net}	Tot. A_{gross}	Tot. R_d	A_{net}	A_{gross}	R_d
2015	YLY900	12±2.4a	49±2.8a	37±1.5a	0.035±0.003a	0.147±0.011b	0.111±0.013a
	CY1000	7.2±1.3b	43.6±7a	36.4±7.3a	0.015±0.004b	0.091±0.005c	0.075±0.005b
	SY63	11.8±2.2a	42.8±2.7a	31±2.8a	0.051±0.012a	0.185±0.014a	0.133±0.007a
	9311	10.9±1.4ab	46±3.4a	35±3.9a	0.044±0.009a	0.182±0.007ab	0.138±0.003a
	XS134	2.7±1.1c	18.1±3.4b	15.4±2.5b	0.012±0.005b	0.078±0.022c	0.067±0.017b
2016	YLY900	7.3±2.4b	47.5±4.1a	40.2±5.6ab	0.021±0.007c	0.136±0.011b	0.115±0.014b
	CY1000	6.9±2.2b	44.3±4ab	37.4±4.6abc	0.017±0.006cd	0.106±0.013c	0.089±0.01bc
	SY63	15±3a	49.6±4.3a	34.6±5.3bcd	0.075±0.016a	0.248±0.021a	0.172±0.023a
	9311	11.9±2.6ab	53.6±3.9a	45.5±4.5a	0.052±0.009b	0.243±0.005a	0.205±0.021a
	XS134	-3.3±3.4c	17.1±4.4c	20.3±6.4e	-0.014±0.014e	0.075±0.018de	0.089±0.026bc
	YY538	-0.4±2.4c	25.8±4.7c	26.2±4.1de	-0.001±0.006de	0.06±0.009e	0.062±0.011c
	YY17	9.3±3.1ab	36±5.8b	26.8±5.6cde	0.024±0.009c	0.091±0.01cd	0.068±0.01c

Abbreviations: Tot. A_{net} , net photosynthetic rate of a whole panicle (nmol s^{-1}); Tot. A_{gross} , gross photosynthetic rate of a whole panicle (nmol s^{-1}); Tot. R_p , respiratory rate of a whole panicle (nmol s^{-1}); A_{net} , net panicle photosynthetic rate on a spikelet basis (nmol s^{-1}); A_{gross} , gross panicle photosynthetic rate on a spikelet basis (nmol s^{-1}); R_d , panicle respiratory rate on a spikelet basis (nmol s^{-1}).

Table 2. Grain yield related agronomic traits of the rice cultivars at harvest. Data presented are mean values with s.d. (n = 5). Data followed by different letters are statistically different at p -value < 0.05.

Year	Cultivar	SN	PL	SD	TGW	GSR	PW
2015	YLY900	356±24b	28.1±1.5a	13.1±1.4b	22.9±0.7d	0.84±0.07bc	7.4±1b
	CY1000	444±30a	21.8±1.1c	21±2.4a	21.6±0.7d	0.8±0.05c	8.7±0.5a
	SY63	231±21c	27.6±0.9a	8.4±0.6c	28.2±1.3b	0.92±0.04ab	6.4±0.6bc
	9311	234±40c	24.5±1b	9.5±1.3c	30.7±0.4a	0.97±0.02a	7.2±1.1b
	XS134	253±14c	17.1±0.7d	14.8±0.9b	25.2±0.8c	0.74±0.08c	5.7±0.4c
2016	YLY900	359±36b	32.7±0.6a	10.9±0.9c	22.2±4b	0.83±0.09b	7±0.9ab
	CY1000	410±44a	26.5±1c	13.8±2.3b	21.8±0.5b	0.81±0.03b	8.1±0.5a
	SY63	216±21c	30.5±1.2b	6.7±0.5d	29.6±1.1a	0.96±0.01a	6.4±0.4b
	9311	211±20c	27.2±1.3c	8.2±0.3d	30.8±1.6a	0.96±0.02a	6.3±0.7b
	XS134	222±32c	18.2±1.5f	11.2±1.1c	24.1±0.3b	0.79±0.05b	4.7±0.3c
	YY538	420±35a	21.6±0.9e	17.2±0.7a	20.5±1.3b	0.77±0.06b	6.9±0.7ab
	YY17	403±33a	23.9±1.1d	15.5±0.9ab	22.3±1b	0.84±0.08b	7.4±0.6ab

Abbreviations: SN, spikelet number per panicle; PL, panicle length (cm); SD, spikelet density (cm⁻¹); TGW, 1000-grain weight (g); GSR, grain setting rate; PW, panicle dry weight (g).

Figures

Figure 1.

Figure 1

A custom-built panicle photosynthesis chamber (P-chamber) for panicle gas exchange measurements. A, An illustration of design of the P-chamber. B, A top-down view of the inner part of the P-chamber. C, Spectrum of the LED light source. D, The difference between the CO₂ concentration in the P-Chamber and the set CO₂ concentration ([CO₂R]) in the reference pipe at different time after closure of the P-Chamber. E, Recorded photosynthetic gas exchange rate of a rice panicle under a photosynthetic photon flux density of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ followed by dark treatment. Letters in panel A and B: a. Battery; b. Wires; c. Voltage adaptor; d. Switch; e. Mixing fan (inside the chamber body); f. Digital displayed voltmeter; g. Cooling fan; h. LED array; i. Chamber body; j. Hasp; k. Sealing strip; l. Rice panicle; m. Cooling fin; n. Clamp. The horizontal lines in panel D represent mean values of the last 10 data points. The y-axis in panel D is log-scaled.

Figure 2.

Figure 2

The dynamic changes of panicle gross photosynthetic and respiratory rates during grain filling for seven rice cultivars. Panel A and B illustrate the four indica cultivars and the three japonica or japonica-indica hybrid cultivars, respectively. Data measured in 2016. Data presented are mean values with s.d. (n = 5).

Figure 3.

Figure 3

Panicle photosynthetic CO₂ response curve (A) and light response curve (B) of rice cultivar YY17. Data measured in 2016. Data presented are mean values with s.d. ($n = 3$). Abbreviations: C_a , ambient CO₂ concentration; PFD, photon flux density.

Figure 4.

Figure 4

Correlation of panicle photosynthetic gas exchange parameters and grain yield related agronomic traits. Data were combined from 2015 and 2016 experiments shown in Table 1 and Table 2 (n = 12). Abbreviations are defined in the figure. The numbers in the figure are the Pearson correlation coefficients. *, p-value < 0.001; **, p-value < 0.0001.

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