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Evaluation of Protocols for Measuring Leaf Photosynthetic Properties of Field-Grown Rice

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Abstract: Largely due to the heterogeneity of environmental parameters and the logistical difficulty of moving photosynthetic equipment in the paddy fields, effective measurement of lowland rice photosynthesis is still a challenge. In this study, we showed that measuring detached rice leaves in the laboratory can not effectively represent the parameters measured *in situ*. We further described a new indoor facility, high-efficiency all-weather photosynthetic measurement system (HAPS), and the associated measurement protocol to enable whole-weather measurement of photosynthetic parameters of rice grown in the paddy fields. Using HAPS, we can conduct photosynthetic measurements with a time span much longer than that appropriate for the outdoor measurements. Comparative study shows that photosynthetic parameters obtained with the new protocol can effectively represent the parameters in the fields. There was much less standard deviation for measurements using HAPS compared to the outdoor measurements, no matter for technical replications of each recording or for biological replications of each leaf position. This new facility and protocol enables rice photosynthetic physiology studies to be less tough but more efficient, and provides a potential option for large scale studies of rice leaf photosynthesis.
Key words: rice; gas exchange; photosynthetic property; *in situ*; high efficiency all-weather photosynthetic measurement system

Improving photosynthetic efficiency is recognized as a major approach to increase crop yields (Long et al, 2006; Zhu et al, 2010). So far, unfortunately, improving photosynthetic efficiency has not been effectively used in the improvements of crop yields. A number of reasons might be related to these, including emphasizing leaf instead of canopy photosynthesis (Zhu et al, 2012; Song et al, 2016) and the difficulty of conducting large scale measurement of photosynthetic physiology in the fields, which discourage genetic studies of photosynthesis. And therefore, what are the major barriers preventing large scale studies of photosynthetic parameters? Though changes in photosynthetic physiological parameters usually take a few days (Geiger, 1976; Oguchi et al, 2003; Schröder et al, 2005), the instantaneous photosynthetic properties, such as the rubisco activation state, stomatal conductance and operating efficiency of photosystem II, are influenced by the environmental conditions, such as light and temperature (Demmig-Adams et al, 1989; Collatz et al, 1991; Salvucci and Crafts-Brandner, 2004). Unfortunately, stable light environments are usually not available in the fields. The high level of environmental heterogeneities makes it difficult to conduct field photosynthetic physiological measurements, in particular in regions where the weather conditions

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vary frequently. Partially as a result of the high level of environmental variations, stable and reliable measurements of photosynthetic rates are only possible when experiments are conducted between late-morning to mid-afternoon on a sunny day (Xu, 2006), since photosynthetic rates measured in the late-afternoon or on cloudy or overcast day usually are lower and inaccurate (Chen and inaclurate, 2006). Besides the high level of diurnal variability, many rice paddy field conditions can be too demanding to perform field measurements. These limitations make large scale field phenotyping of photosynthetic parameters in a large genetic population difficult to implement.

Growing large panels of crop varieties or accessions in the greenhouse and measuring photosynthetic physiology in the laboratory can be used as an effective option to study the photosynthetic physiology. However, many laboratory and field experiments show that plants grown in the fields show drastic difference in the photosynthetic parameters (Mahon and Hobbs, 1981; Mishra et al, 2012; Kolari et al, 2014). Plants in the fields routinely experience dynamic environments, such as changes in light, temperature and humidity, which can not be easily replicated in the greenhouse. The light quality, nutrition components, and soil depth etc. in laboratory usually cannot meet the growth demand of crop plants such as maize and rice. As a result, the parameters obtained in laboratory can not represent the actual physiological parameters of plants grown in the fields, even without considering that practical space limitation of growing large genetic populations in a greenhouse.

An indoor gas exchange measurement method commonly used for field-grown plants is to use leaves detached from the fields. Driever et al (2014) conducted large scale photosynthetic measurements of field-grown wheat in the laboratory using detached tillers. Leakey et al (2006) also measured photosynthetic carbon dioxide response curve (*A*-C_i curve) and photosynthetic light response curve (*A*-*Q* curve) with excised leaves of maize. However, the feasibility of the method in rice has never been evaluated. In this study, we first explored the feasibility of the protocol that measuring photosynthesis in detached rice leaves. Furthermore, to overcome difficulties in accurate and high-throughput characterization of photosynthetic parameters for field-grown rice, we designed a new indoor measurement facility high-efficiency all-weather photosynthetic measurements system (HAPS) and a related indoor measurement protocol.

MATERIALS AND METHODS

Field management

Field experiments were conducted at Songjiang breeding station (30º56′44″ N, 121º8′1″ E) of the Shanghai Institutes of Plant Physiology and Ecology, Shanghai, China. A japonica rice variety Xiushui 134 was used. Seeds after germination were sown on seedbeds in the field on 1 June, 2015, and seedlings were transplanted to field on 26 June, 2015. One plant was transplanted into each hill at a planting density of 25 hills/m^2 (0.20 m × 0.20 m). The rice plants started rapid shoot elongation on 10 August and flowering on 8 September, and were harvested on 28 October, 2015. Nitrogen fertilizer was applied at a rate of 200 kg/hm^2 with 35%, 35% and 30% of the total nitrogen fertilizer applied at the pre-transplanting, tillering and booting stages, respectively. Phosphate (P_2O_5) and potassium (K_2O) fertilizers were both applied as basal fertilizers before transplantation at the rate of 150 kg/hm^2. Weeds, pests and diseases were controlled periodically with herbicides, insecticides and fungicides.

Leaf gas exchange measurements

A-C_i and *A*-*Q* curves were measured weekly with a LI-6400 infrared analyzer (Li-Cor Inc., Lincoln, Nebraska, USA) after 30 July, 2015. Before indoor measurements, plants were dug from the field. Two neighboring plants were dug out together with adhesive soil and put into a bucket. Four buckets (eight plants) were sampled each time for each treatment. Plants were placed in the measurement facility for one-day adaptation [a level of 1 800 μmol/(m^2·s) artificial light was turned on at 6:30 am and turned off at 18:00 pm], after which measurements were conducted. For both outdoor and indoor measurements, plants located at the edge of rows or columns were not used to avoid boundary effects.

For *A*-C_i curve measurements, we first set the light intensity being 1 800 μmol/(m^2·s), air flow rate being 500 μmol/s, leaf chamber block temperature being 30 ºC, which matched ambient air temperature and was suitable for rice leaf physiological activity, and reference CO_2 concentration being 425 μmol/mol, and maintained leaves under such a condition for 15 min for adaptation and stabilization of leaf photosynthesis. Then, we used the following reference CO_2 concentrations 425, 350, 250, 150, 100 and 50 μmol/mol for 2.5 min