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【Short Report】

Correlation of Chlorophyll Meter Readings with Gas exchange and Chlorophyll Fluorescence in Flag Leaves of Rice (*Oryza sativa* L.) Plants

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Abstract : The objective of this study was to establish the correlation of the chlorophyll meter (SPAD) readings with the contents of chlorophyll (Chl) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the gross photosynthetic rate (P_G), and the maximum quantum yield of photosystem II (PSII) (F_v/F_m) in flag leaves of rice (*Oryza sativa* L.) in ripening stage. The SPAD readings significantly correlated with the Chl content, the Rubisco content, P_G and F_v/F_m (R^2=0.848, 0.648, 0.671 and 0.712, respectively), which suggests that the SPAD meter has the potential to estimate the photosynthetic capacity of the flag leaves. However, both P_G and F_v/F_m had a stronger relationship with the Rubisco content than the SPAD readings, indicating that the PSII photochemical and CO_2 assimilation capacities are strongly influenced by the Rubisco content. Therefore, accurate calibration would be indispensable to obtain the physiological information from the SPAD readings of flag leaves.

Key words : Chlorophyll fluorescence, Chlorophyll meter, Flag leaf, Photosynthesis, Rice, Rubisco.

The yield of major crops including rice has increase by about two-fold in the last century through genetic improvement, control of pests and diseases, and greatly increased application rate of nitrogen (N) fertilizer (Evans, 1993). However, further increase is needed to meet demand of the future populations (Mitchell and Sheehy, 2006). The improvement of leaf photosynthesis is one of the fundamental steps in increasing the yield of rice. Several researchers reported that a series of rice cultivars released in Japan over a 100-year period showed yield improvements, and concluded the photosynthetic improvements of flag leaves could be seen two to three weeks after heading (Sasaki and Ishii, 1992 ; Zhang and Kokubun, 2004). From previous findings, it may be predicted that improvements of flag leaf photosynthesis help increase the future grain yield potential.

The chlorophyll (Chl) meter (SPAD meter) is a portable, simple, quick and nondestructive tool that measures the Chl content of the leaves. The leaf Chl content is affected by N status of the plant. Therefore, the SPAD meter has been used to predict the need for additional N fertilizer in rice plants (Peng et al., 1996). The role of Chl in photosynthesis was well established. A highly significant correlation of the SPAD readings with the photosynthetic rate was obtained in soybean (*Glycine max* (L.) Merr.) (Ma et al., 1995) and weed species (*Amaranthus vlitus* L.) (Kapotis et al., 2003). However, a correlation of the SPAD readings with the photosynthetic parameters of the rice leaves has not been reported. The correlation analysis of the SPAD readings with the photosynthetic parameters of the rice leaves may be important to make the advanced interpretations of data from the SPAD meter. This information will contribute to improvements of leaf photosynthesis in rice cultivars.

Leaf photosynthesis consists of the several physiological processes, that is, light harvesting, photosystem II (PSII) photochemistry, and CO_2 assimilation. Moreover, the photosynthetic capacity is affected by various environmental factors, for example, irradiance, temperature, humidity, and N conditions. Therefore, the photosynthetic capacity may not be predicted solely by the SPAD readings. In this study, we investigated if there was a correlation of the SPAD readings with the Chl content, and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content, photosynthesis and Chl fluorescence, in the flag leaves of rice plants in the ripening stage.

Materials and Methods

Two *japonica* cultivars (Shirobeniya and Nippon-

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Abbreviations : Chl, chlorophyll; F_o, initial Chl fluorescence of a dark-adapted leaf; F_m, the maximum Chl fluorescence of a dark-adapted leaf; F_v, variable Chl fluorescence; N, nitrogen; NPT, new plant type; P_G, gross photosynthetic rate; PPFD, photosynthetic photon flux density; PSII, photosystem II; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

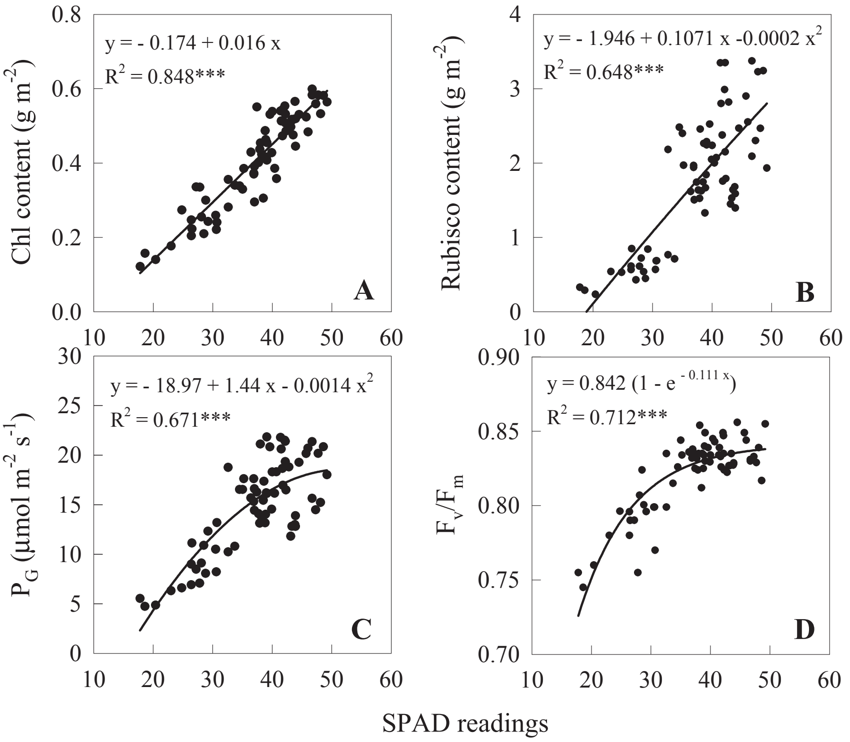

Fig. 1. Correlation of the SPAD readings with the Chl content (A), the Rubisco content (B), the gross photosynthetic rate (P_G, C), and the maximum quantum yield of PSII (F_v/F_m, D) in the flag leaves of rice plants. *** indicates a significant correlation at the 0.1% level.

bare), a *japonica-indica* intermediate type (Akenohoshi), a new plant type line (BSI429), and an *indica* cultivar (IR36) were used in this experiment. Water-soaked seeds of these cultivars were sown in nursery boxes in a glasshouse in summer season, 2006. After three weeks, young seedlings were transplanted to water bathes of 500 L capacity. This water bath contained the nutrient solution according to Yoshida et al. (1972). De-ionized water was used to make up the solution. The seedlings were grown in a glasshouse with a temperature from 22±0.5ºC at midnight and to 33 ± 0.4ºC at midday under natural sunlight. They were divided into N-sufficient and N-deficient groups and thereafter were grown in standard (2.86 mM of N) and low (1.43 mM of N) nutrient solutions, respectively. Each solution was renewed at a two-week interval, and the pH of the solution was adjusted every day to 5.0–5.5. The solution renewal method was as described by Kumagai et al. (2007) with slight modification. To obtain the correlation of a large range of the SPAD readings with photosynthetic parameters, we measured the parameters using the flag leaves at different ages of the five rice cultivars grown at the two N levels.

The mean of three SPAD readings from the SPAD meter (SPAD-502, Konica Minolta Sensing Co., Japan) was obtained around the midpoint of each leaf blade. After readings, the gas exchange rate was measured with an open system using a temperature-controlled chamber under the following conditions: leaf temperature, 30±0.4ºC; CO_2 concentration, 380±13 μL L^{-1}; relative humidity, 60±2.6%; and photosynthetic photon flux density (PPFD), 1000 μmol m^{-2} s^{-1}. The leaf area used for the measurements was 5.9 cm^2, and the rate of airflow into the assimilation chamber was 706 μmol s^{-1}. The CO_2 concentration and water vapour pressure in the reference and sample air were monitored with an infrared gas analyzer (Li-6262, LI-COR, USA). Based on the measurement values, the gross photosynthetic rate (P_G) was calculated as described by Kumagai et al. (2007). The Chl florescence of PSII was monitored by using a portable fluorometer (PAM-2000, Waltz, Germany). Using a leaf that was dark-adapted for 30 min, the initial fluorescence (F_0) in non-photosynthetic conditions was determined with low intensity of a measuring beam; thereafter, the maximal fluorescence (F_m) was measured by applying a 0.8-s saturation pulse onto the leaf in order to reduce all the PSII centres. Based on the data obtained, the maximum quantum yield of PSII (F_v/F_m) was calculated according to the method described by van Kooten and Snel (1990). After the gas exchange and Chl fluorescence were measured, leaf discs of 5-mm diameter were sampled, frozen in liquid N_2, and stored at –80ºC. The contents of Chl and Rubisco were measured as described by Kumagai et al. (2007).

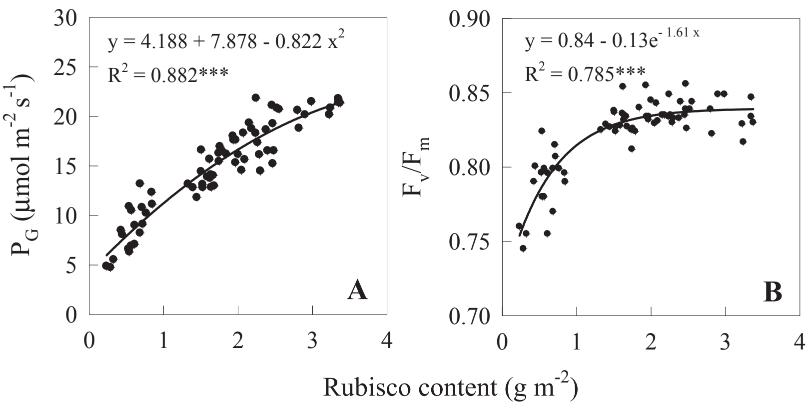

Fig. 2. Correlation of the Rubisco content with the gross photosynthetic rate (P_G, A), and the maximum quantum yield of PSII (F_v/F_m, B) in the flag leaves of rice plants. *** indicates a significant correlation at the 0.1% level.

Results and Discussion

As shown in Fig. 1A, the relationship between the SPAD readings and the extracted Chl content was linear with the high regression coefficient (R^2=0.848). A relationship between the SPAD readings and the leaf Chl content has been established for several crop species (Yamamoto et al., 2002 ; Netto et al., 2005 ; Fritschi and Ray, 2007), and the regression model was different among species in the reports. According to the report by Takebe and Yoneyama (1989), the regression line for the SPAD readings and the Chl content in rice was significantly linear. This result was in agreement with ours.

It is notable that the correlation of the SPAD readings with the amount of Rubisco was curvilinear with a high coefficient (R^2=0.648) (Fig. 1B).The SPAD readings higher than 30 showed a poor relationship for the Rubisco content. This poor relationship is due to the difference in the reduction pattern between the Chl and Rubisco content during the leaf senescence. In rice leaves, the Rubisco content markedly decreased with a slight decrease in the Chl content during the leaf senescence (Makino et al., 1983 ; Huang et al., 2004).

We observed that the relationship between the SPAD readings and P_G was curvilinear with the high regression coefficients (R^2=0.671) (Fig. 1C). The SPAD meter can be used to monitor the leaf N content in rice plants (Takebe and Yoneyama, 1989). The leaf N content has commonly a strong positive relationship with photosynthetic rate, as reviewed by Sinclair and Horie (1989). Therefore, a good correlation of the SPAD reading with P_G was also obtained in our study. Thus, we propose that the SPAD meter has the potential to estimate the photosynthetic capacity of the flag leaves of rice cultivars during the ripening stage.

The measurements of Chl fluorescence was widely used in investigating the functional situation of photosynthetic system under the various stress conditions. However, there was no attempt to analyze the correlation of the SPAD readings with Chl fluorescence of rice leaves so far. An exponential mathematical model best fitted the relationship between the SPAD readings and the F_v/F_m ratio (R^2=0.712), as shown in Fig. 1D. F_v/F_m was positively related to the quantum yield of O_2 evolution in photosynthesis, and the values of 0.800±0.05 corresponded to the high efficiency of excitation energy in PSII (Björkman and Demmig, 1987). According to this fitted regression model, the SPAD readings around 30 indicate the beginning of possible impairment of PSII. Thus, we propose the use of the SPAD readings as an indicator of stress in the flag leaves of rice plants.

In general, the decrease of CO_2 assimilation capacity is usually associated with the decrease in Rubisco content of rice leaves (Murchie et al., 2002 ; Kumagai et al., 2007). As shown in Fig. 2A, there was the highly curvilinear relationship between the Rubisco content and P_G (R^2=0.882). Moreover, we observed an exponential relationship between the Rubisco content and the F_v/F_m ratio (R^2=0.785) (Fig. 2B). The F_v/F_m ratio was declined rapidly when the Rubisco content decreased below 1.0 g m^{-2}, which indicates that decreased CO_2 assimilation capacity induced by the decrease in the Rubisco content potentially lead to an over-reduction of the photosynthetic electron transport chain and therefore photoinhibition in PSII. Both P_G and F_v/F_m have the stronger relationships with the Rubisco content than the SPAD readings, indicating that the PSII photochemical and CO_2 assimilation capacity are strongly influenced by the Rubisco content. Several researchers reported that the contents of Chl and Rubisco and their balance of rice leaves were affected by irradiance (Murchie et al., 2002 ; Chen et al., 2003). This fact indicates that the regression line of the SPAD readings to P_G and F_v/F_m may be different between the rice plants grown at different levels of irradiances. Thus, we must apply the correct calibration when the photosynthetic capacity is physiologically assessed from the date of the SPAD meter.

Our result showed good correlations of the SPAD readings with some photosynthetic parameters, which suggests that the SPAD readings are possibly an indirect indication of the photosynthetic capacity in the flag leaves of rice cultivars during the ripening stage. However, importantly, the accurate calibration would be indispensable to obtain the physiological information from the SPAD readings of flag leaves.

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