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BRIEF COMMUNICATION

Underestimate of PS2 efficiency in the field due to high leaf temperature resulting from leaf clipping and its amendment

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Abstract

Chlorophyll fluorescence parameter F_v/F_m, an indicator of the maximum efficiency of PS2, is routinely measured in the field with plant leaves darkened by leaf clips. I found that on a sunny day of subtropical summer, the F_v/F_m ratio was often underestimated because of a large F_0 value resulted from a high leaf temperature caused by clipping the leaf under high irradiance, especially for long (*e.g.* 20 min) duration. This phenomenon may overestimate the down-regulation of PS2 efficiency under high irradiance. When leaf temperature was lower than 40 °C, the F_0 level of rice leaves under clipping remained practically unchanged. However, F_0 increased drastically with leaf temperature rising over 40 °C. In most measurements, no significant difference in F_m was found between rice leaves dark-adapted by leaf clips for 10 min and for 20 min. Therefore, shading leaf clips to prevent a drastic increase of leaf temperature, using F_0 measured immediately after the leaf being darkened to calculate F_v/F_m, as well as shortening the duration of leaf clipping are useful means to avoid an underestimate of F_v/F_m.

Additional key words: chlorophyll fluorescence; leaf clip; leaf temperature; irradiance; *Oryza*; photosystem 2 efficiency; rice.

Chlorophyll (Chl) fluorescence parameters are widely used as indicators of functional changes of photosynthesis apparatus (Ball *et al.* 1994, Roháček and Barták 1999, Thomas and Turner 2001). Among these parameters, the ratio between light-induced variable and maximum fluorescence of dark-adapted leaves (F_v/F_m) is an indicator of the potential photochemical efficiency of photosystem 2 (PS2), and F_v is defined as the difference of maximal and minimal fluorescence ($F_v = F_m - F_0$) of dark-adapted leaves (Ball *et al.* 1994, Roháček and Barták 1999).

The efficiency of PS2 is influenced by environmental factors. In habitats fully exposed to sun, leaves may absorb more photons than that they can utilize. Under some environmental stresses, excessively absorbed photons may increase the stress-induced decrease in photosynthetic capacity (Demmig-Adams and Adams 1992, Long *et al.* 1994, Osmond and Grace 1995), because reactions of Calvin cycle are affected to a greater extent than the processes of photon absorption and electron transport (Baker 1994, Leegood 1995).

In order to understand the effects of environmental stresses on PS2 efficiency, measurements of F_0 and F_m are often taken in the field using leaf clips to darken the leaves. Transpiration is a major mechanism for withdrawing heat from the leaf to cool it off. When a leaf is covered with leaf clip, its temperature may rise under the sun due to a block of transpiration. Many researchers have pointed out that heating may change the Chl fluorescence intensity (Downton *et al.* 1984, Seemann *et al.* 1986, Havaux *et al.* 1988, Nauš *et al.* 1992, Kitao *et al.* 2000, Braun *et al.* 2002, Knight and Ackerly 2002). Among Chl fluorescence, F_0 is influenced readily by high temperature, and this change in F_0 occurs in two steps: firstly F_0 varies slightly between 20 and 40 °C, and then rises sharply at 40–50 °C (Kitao *et al.* 2000, Braun *et al.* 2002, Knight and Ackerly 2002). The temperature at which F_0 starts to increase sharply (T_c) is varied with species and environmential conditions; species or plants growing in warmer or drought conditions always have higher T_c (Downton *et al.* 1984, Seemann *et al.* 1986, Havaux *et al.* 1988, Knight and Ackerly 2002).

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Abbreviations: Chl – chlorophyll; F_0 – basic chlorophyll fluorescence; F_m – maximum chlorophyll fluorescence; F_v/F_m – potential efficiency of PS2; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; T_c – temperature at the start of F_0 sharp increase.

However, my previous work (Weng and Lai 2005) found that F_0 of many subtropical- and tropical-origin C_3 species, including orange, mango, rice, and sweet potato, increased sharply at temperatures of *ca*. 25–40 °C. Therefore, on hot and clear days, F_v/F_m of these species might be underestimated due to this high F_0 value, obtained at high leaf temperature when the leaf is covered with a clip. The aim of the present study was to elucidate the effects of temperature on F_0 and F_m of rice leaves in the field, and to find means to minimize the impact of high temperature on fluorescence measurements when leaves were clip-covered.

Attached, fully expanded youngest leaves of field-grown rice (*Oryza sativa*, cv. Taiken 14) on the campus of National Chung-Hsing University, Taichung, Taiwan (24°10'N, 78 m) were used as materials. At maximum tillering stage of rice (August 2004), Chl fluorescence parameters (F_0 and F_m) and leaf temperature were measured every 30 min from 09:00 to 18:00 (local time) on a sunny day. The fluorescence parameters were measured with a portable fluorometer (*Handy PEA*, *Hansatech*, UK) after 0, 10, and 20 min dark adaptation with leaf clips. Both F_0 and F_m were measured with saturating pulses of 1 500 μmol m^{-2} s^{-1}. Leaf temperature was taken with a radial thermometer (*TA-0510bF, Minolta*, Japan) before each measurement of Chl fluorescence, and with copper-constantan thermocouples connected to the abaxial surface of the leaves (Weng and Lai 2005) inside leaf clips. The photosynthetic photon flux density (PPFD) of solar radiation was monitored with a quantum meter (*LI-185A*, *Li-Cor*, USA).

From 09:00 to 18:00 h, 18 measurements were made with leaf clips at 6 time intervals (at 10:00, 11:00, 12:00, 13:30, 14:30, and 16:00) shaded by *Styrofoam* plates to avoid heating of the leaves from sun exposure and those of the remaining measurements exposed to sunlight. Four leaves selected from 4 plants were used in each measurement, with each leaf being measured 3 times. The average of each leaf was used as the statistical parameter of each replication.

Results of the present study showed that on a clear summer day in subtropical region, leaf temperature before being darkened with a leaf clip was higher (*ca*. 35 °C) at midday and lower (*ca*. 30 °C) at early morning and dusk (Fig. 1*B*). Leaf temperature increased sharply (Max. +7 °C) when they were covered with leaf clips under high irradiation. On the contrary, the rise of leaf temperature was moderate (less than +3.5 °C) when clips were shaded or under low (<800 μmol m^{-2} s^{-1}, PPFD) irradiation (Fig. 1*A,B*).

Our previous report (Weng and Lai 2005) indicated that, when leaves were heated at the rate of *ca*. 1 °C min^{-1} in darkness, the level of F_0 was strongly dependent on leaf temperature at temperature higher then *ca*. 27 °C in winter and 43 °C in summer. Fig. 1*C* shows that F_0 remained relatively stable when leaf temperature was below the critical temperature (T_c = 40 °C); and it increased sharply when temperature was higher than T_c. The F_0 rise was slight when the leaf was clipped for 10 min, and more serious rise was observed after 20 min (Fig. 1*C*). I found that if leaf temperature reached 40 °C and more, F_0 increased, especially 20 min after clipping.

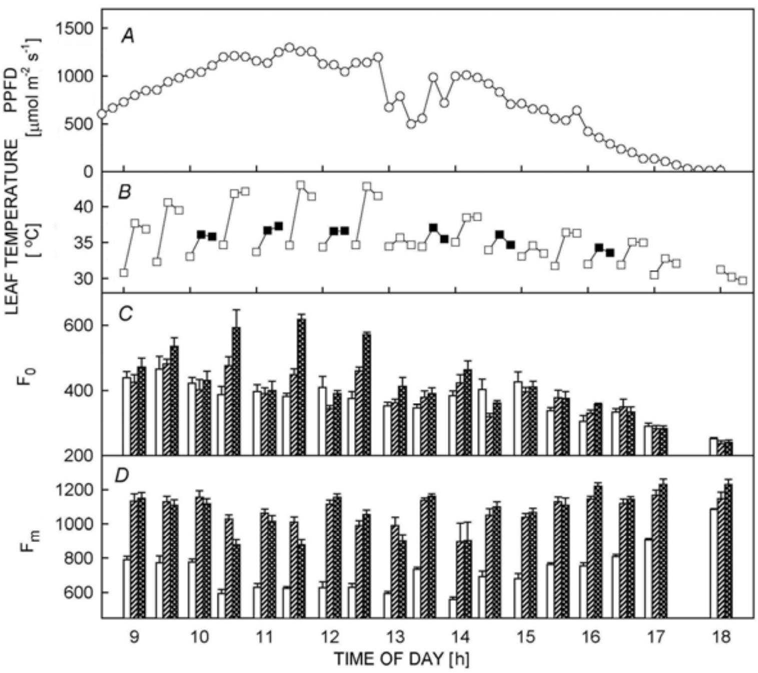

Fig. 1. Diurnal variation of photosynthetic photon flux density (PPFD) of solar radiation; leaf temperature (□: under irradiation, ■: leaf clips were shaded with *Styrofoam* plate), and maximal and minimal chlorophyll fluorescence (F_m and F_0) taken immediately after (*open bars*), 10 min (*diagonal-marked bars*) and 20 min (*crossed bars*) after the leaves were darkened with leaf clips.

Usually light-adapted leaves showed lower maximal fluorescence (F_m') than dark-adapted leaves (F_m), due to fluorescence quenching relax in darkness (Roháček and Barták 1999). Fig. 1*D* shows that F_m taken immediately after darkening was of course significantly lower than that taken 10 and 20 min after the leaves were covered with clips. However, there was no significant difference between F_m of rice leaves dark-adapted for 10 and 20 min in most measurements (Fig. 1*D*).

The excess energy absorbed by plant leaves can often lead to a reduced efficiency of PS2 (Demmig-Adams and Adams 1992, Long *et al*. 1994, Osmond and Grace 1995). Reports have pointed out that under high irradiance of midday, many plants down-regulated their PS2 efficiency, assessed as F_v/F_m. This was mainly due to a decline of F_m (Joshi 1995, Faria *et al*. 1996, Xu and Wu 1996) or both a decline of F_m and an increase of F_0 (Joshi 1995, Xu and Wu 1996). The same tendency for the diurnal variation of F_m as just mentioned (Joshi 1995, Faria *et al*. 1996, Xu and Wu 1996) was observed in the present study (Fig. 1*D*). However, Fig. 1 shows that F_0 slightly declined, instead of increased, at midday when data of high leaf temperature (>40 °C) were excluded. Therefore, the down-regulation of F_v/F_m in rice leaves at midday was primarily due to the decline of F_m when leaf temperature was lower than 40 °C, *i.e.* under clouds or with shaded leaf clip. Yet, Fig. 2*B* shows a drastic decline of F_v/F_m (by *ca.* 0.3) when leaves were darkened with leaf clips under high irradiance, especially at long duration (20 min). This was due to a rapid increase of F_0 when leaf temperature was higher than T_c. Thus, under high irradiance, the F_v/F_m was underestimated, and consequently the effect of irradiance on PS2 efficiency was overestimated. Shading of leaf clips could prevent the drastic increase of leaf temperature and F_0 as well, maintaining a higher F_v/F_m even at midday (Fig. 2*A,B*). In addition, there was no significant difference among F_0 measured immediately afterwards, as well as 10 and 20 min after darkening the leaf with a clip, when leaf temperature did not reach 40 °C (Fig. 1*B,C*). This result indicates that F_0 of rice leaves was not affected by the duration of dark-adaptation with leaf clip when leaf temperature remained lower than T_c. Therefore, using F_0 measured immediately after covering the leaf with a clip, instead of the F_0 measured 10 or 20 min after the covering, is another way to avoid an underestimate of F_v/F_m due to a high F_0 caused by high leaf temperature.

Fig. 2*A*,*B* shows that under high irradiance the F_v/F_m ratio, calculated from F_0 taken immediately after clipping, was much higher than that calculated from F_0 taken 10 and 20 min after clipping the leaf. This F_v/F_m was close to the values obtained with shaded clip 30 min before or 30 min later (Fig. 2*A*,*B*). Besides, there was no significant difference between F_m of rice leaves dark-adapted for 10 and 20 min in most measurements (Fig. 1*D*). In view of a larger increase of F_0 for a leaf clipped for 20 min than for a leaf clipped for 10 min, shortening the duration of darkening with leaf clip is an important process to avoid the underestimate of F_v/F_m measured in the field under high irradiance.

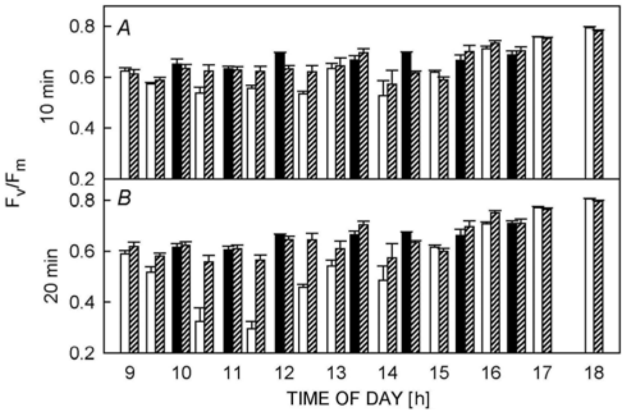

Fig. 2. Comparison of F_v/F_m calculated using various F_0's. *Open and solid bars*: F_v/F_m calculated from F_0 taken 10 or 20 min after the leaves were darkened with leaf clips under irradiation and shaded with *Styrofoam* plate, respectively. *Diagonal-marked bars*: F_v/F_m calculated from F_0 taken immediately after the leaves were covered with leaf clips.

Our previous paper (Weng and Lai 2005) indicated that many subtropical and tropical origin C_3 species had lower T_c (*ca*. 25–40 °C). In this work, I found that on a sunny day of subtropical summer, leaf temperature of rice rose above T_c readily when the leaf was darkened with clip under high (>800 µmol m^{-2} s^{-1}) irradiance (Fig. 1*A*,*B*). Therefore, F_v/F_m was underestimated easily because of a high F_0. I suggest that shading leaf clips to prevent a drastic increase of leaf temperature, use of F_0 measured immediately after leaf darkening to calculate F_v/F_m, as well as diminishing the duration of clipping are useful means to avoid the underestimation of F_v/F_m.

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