Determination of Maximal Chlorophyll Fluorescence Using A Multiphase Single Flash of Sub-Saturating Intensity

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INTRODUCTION

- It is necessary to determine maximal fluorescence (Fm') in order to estimate the effective quantum efficiency of Photosystem II (Φ_{PSII}) and the rate of electron transport (ETR) from chlorophyll fluorescence measurements (Genty *et al.*, 1989).
- Fm' is commonly measured using a single saturating, multi-turnover flash of 400-1200 ms duration to reduce the Q_A-PQ pool. In many conditions, full reduction of the Q_A-PQ pool cannot be achieved using such a protocol, especially in field plants.
- Here we present a 'multiphase' single flash method (MSF) to estimate the true maximal fluorescence level in these conditions.

MATERIALS & METHODS

- With light emitting diode (LED) technology, "saturating" light can be supplied in several different ways, which are described in **Figure 1**.
- LI-6400 Portable Photosynthesis Systems with 6400-40 Leaf Chamber Fluorometers (LI-COR, Lincoln, NE, USA) were used to compare the three methods. Chamber conditions were maintained at ambient levels of CO_2, relative humidity, and temperature.
- Φ_{PSII} was calculated as: 1 – Fs/Fm', where Fs is steady state fluorescence and Fm' corresponds to maximal fluorescence. ETR was calculated as: Φ_{PSII}*f*Q*α_{Leaf}. The fraction of absorbed quanta used by PSII, f, was assumed to be 0.5; incident photon flux density, Q, was measured with an internal PAR sensor; and maize leaf absorptance, α, was measured as 0.85.

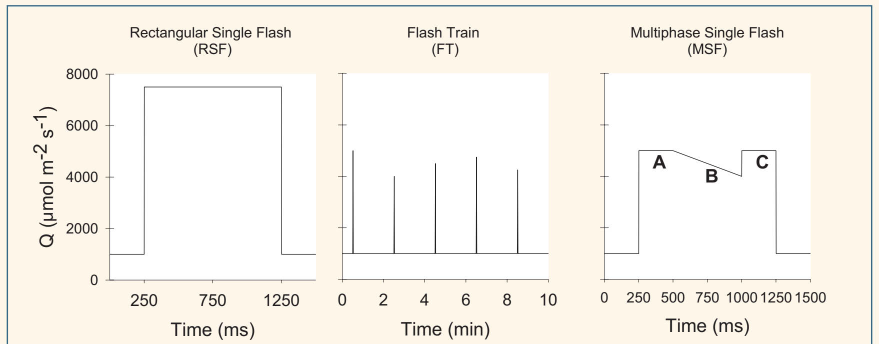

Figure 1. Methods to determine maximal fluorescence (Fm'). *Rectangular Single Flash* method (RSF): a saturating multiturnover flash (Q) of 400-1200 ms duration. *Flash Train* method (FT): applies five rectangular flashes of various Q in random order, separated by two minutes. *Multiphase Single Flash* method (MSF): (A) high, nearly saturating Q for 250 ms to reduce Q_A-PQ pool; (B) ramp of declining Q for 500 ms; (C) return to the initial high Q for 250 ms to check for flash-induced non-photochemical quenching (qN). Fm' values from phase B of the MSF method and each flash of the FT method are regressed against 10^4/Q and extrapolated to estimate the maximal fluorescence at infinite flash intensity.

Table 1. Comparison of Fm', Φ_{PSII}, and ETR in field-grown maize leaves measured by RSF and FT methods. Data are computed as [(RSF – FT)/FT) x 100%] for each of the variables on each of n leaves.

Incident PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	n	Fm'	Φ_{PSII}, ETR
		---% *difference*---	
250	9	-19**	-8**
500	11	-16**	-10**
1000	14	-20**	-19**
1500	13	-22**	-28**
2000	14	-16**	-41**

** Significant at α0.01

Table 2. Comparison of Fm', Φ_{PSII}, and ETR in field-grown maize leaves measured by FT and MSF methods. Data are computed as [(MSF – FT)/FT) x 100%] for each of the variables on each of n leaves.

Incident PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	n	Fm'	Φ_{PSII}, ETR
		---% *difference*---	
250	9	2^{ns}	3^{ns}
500	11	6^{ns}	2^{ns}
1000	14	4^{ns}	4^{ns}
1500	13	4*	8*
2000	14	6^{ns}	4^{ns}

^{ns} Significant at α0.05 *Significant at α0.05

THE MULTIPLE FLASH TRAIN SOLUTION

- Markgraf & Berry, 1990 and Earl & Ennahli, 2004, proposed an approach using a multiple flash train (FT) of variable light intensities (Q) to estimate the true Fm' (**Figure 1**).
- **Table 1** shows the rectangular single flash (RSF) method under-estimates Fm' compared to the FT method at all light intensities in field-grown maize leaves.
- However, the FT method requires multiple fluorescence measurements at different light pulse intensities, with wait times between each measurement, to obtain one valid estimate of Fm', Φ_{PSII} and ETR, which takes about 10 minutes. This is time-consuming and laborious if many measurements are to be made, and is especially difficult under rapidly changing environmental and physiological conditions.

THE NEW "MULTIPHASE" SINGLE FLASH SOLUTION

- The MSF method uses a ramp of rapidly varying light intensity to generate Fm' values over a range of light intensities (**B, Figure 1**).
- Total duration of one MSF measurement is only about one second.

Validation with DBMIB

- As a first step to evaluate the MSF method, we vacuum infiltrated leaves of *P. sativum* with 150 µM DBMIB to restrict turnover of the PQ pool and provide conditions in which nearly full saturation could be assured.
- The fluorescence response during the ramp phase (**Panel II Inset, Figure 2**) has a hyperbolic shape. The y-intercept of a plot of the fluorescence response as a function of 1/Q (**Panel II, Figure 2**) is used to estimate the maximal fluorescence at infinite Q.
- Fm' measured by the RSF method was within 1% of the extrapolated Fm' value measured by the MSF method (**Panel II, Figure 2**). Thus it should be possible to use the MSF method to derive Fm' in conditions where saturation by the traditional RSF method cannot be achieved.

Validation in field-grown Z. mays

- **Figure 3** illustrates the new MSF method in field-grown maize. In this case, the traditional RSF method gave a 17% lower estimate of Fm' than the extrapolated value predicted by the MSF method.
- **Table 2** and **Figure 4** show that the FT and MSF methods gave similar estimates of Fm', Φ_{PSII} and ETR at all light levels.
- **Figure 5** shows that ETR calculated by both the new MSF and FT methods are proportional to gross CO_2 assimilation at all irradiances, as expected for C4 plant metabolism; however, ETR estimated using the traditional RSF method increasingly underestimates results from gas exchange as irradiance increases.
- In maize, under-estimation of Fm' by the traditional RSF method can lead to miscalculation of Φ_{PSII} and ETR by as much as -41% at high light intensities. The new multiphase single flash (MSF) method resolves this problem.

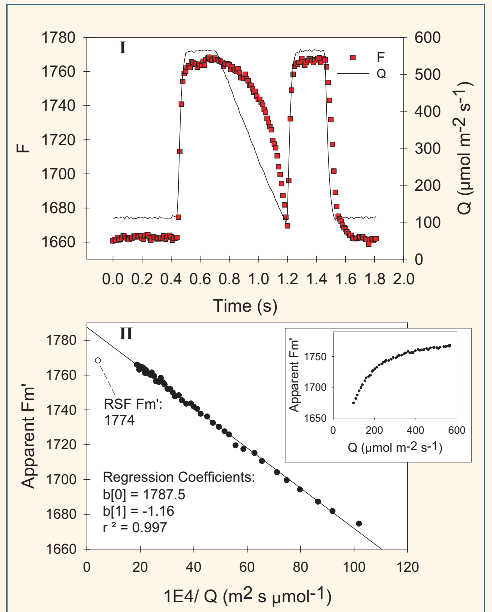

Figure 2. *Panel I.* Fluorescence response to the MSF protocol in a typical leaf of greenhouse-grown *P. sativum* vacuum infiltrated with DBMIB. *Panel II.* Linear regression of Fm' versus 10^4/Q obtained during phase B, Panel I. *Inset:* Fluorescence response to Q during the ramp.

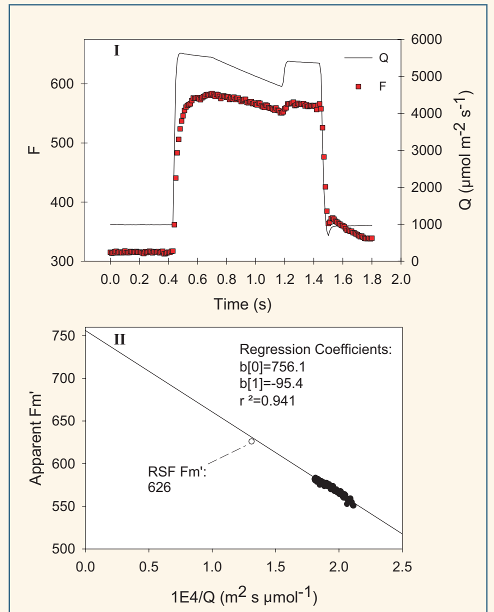

Figure 3. *Panel I.* Typical fluorescence response to the MSF protocol in a field-grown maize leaf at an incident photon flux density of 1,000 µmol $m^{-2}\ s^{-1}$. *Panel II.* Linear regression of Fm' versus 10^4/Q using the ramp data.

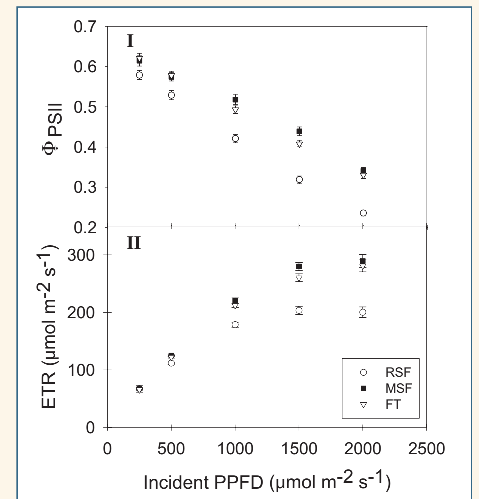

Figure 4. *Panel I.* Φ_{PSII} measured in field-grown maize at growth stage V-5 as a function of light intensity. *Panel II.* PSII electron transport rate (ETR) computed from the data in Panel I versus light intensity. Each data point is the mean of 9 to 14 observations. Error bars indicate standard errors of the means.

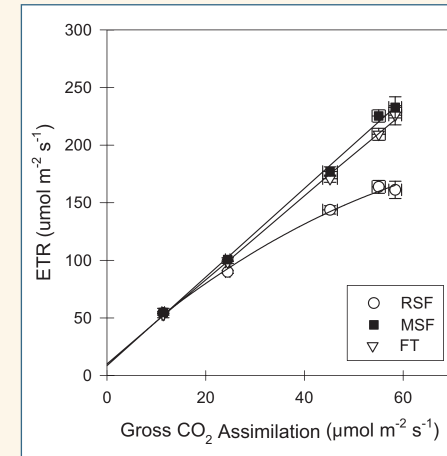

Figure 5. Comparison of ETR calculated using the RSF, FT and MSF methods to gross CO^2 assimilation rate measured from gas exchange in field-grown maize at growth stage V-5.

CONCLUSIONS

- The new multiphase single flash method can be used to derive true estimates of Fm', Φ_{PSII} and ETR in a flash time duration of approximately 1s.
- The traditional rectangular saturating flash method leads to large under-estimates of Fm', Φ_{PSII} and ETR, especially at higher light intensities.
- For the first time, this new approach allows both rapid and valid determination of the rate of photosynthetic electron transport in conditions where Fm' is difficult to saturate, or when leaf physiological and environmental status is changing rapidly (e.g. field conditions).

REFERENCES

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