A new multiphase single flash method of determining Fm' is important for field-grown plants in order to accurately calculate Φ_{PSII} and the rate of electron transport (J) using chlorophyll fluorescence measurements

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Introduction

- Maximal fluorescence (Fm') is needed to estimate the effective quantum efficiency of Photosystem II (Φ_{PSII}) from chlorophyll fluorescence measurements (Genty et al., 1989)
- Fm' is commonly measured using a constant, saturating flash of light of 400-1200 ms duration (rectangular flash, RF) (Figure 1) to fully reduce the primary acceptors of Photosystem II
- The RF protocol does not always completely reduce the primary acceptors of Photosystem II, especially in field plants (Markgraf and Berry, 1990; Earl and Ennahli, 2004; Loriaux et al., 2006)
- A new multiphase single flash (MPF) is a promising alternate fluorescence protocol that may be used to find the true Fm' in difficult-to-saturate conditions (Figure 2)

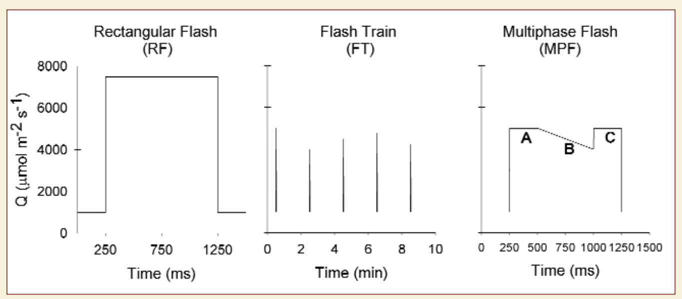

Figure 1. Methods to determine maximal fluorescence (Fm')

Rectangular Single Flash method (RF): a saturating 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 Flash method (MPF): high, nearly saturating Q for 250 ms (A); ramp of declining Q for 500 ms (B); return to the initial high Q for 250 ms (C). Fm' values from phase B of the MPF method and each flash of the FT method are regressed against 1/Q and extrapolated to estimate the maximal fluorescence at infinite flash intensity.

Aim of this Work

1. Test the extent that the rectangular flash (RF) method underestimates Fm' under field conditions
2. Test the performance of a new multiphase flash (MPF) technique in providing a rapid and reliable Fm' measurement under field conditions

Experiment

- The rectangular flash (RF) and multiphase flash (MPF) methods (Figure 1) were compared under both easy- and difficult-to-saturate conditions using combined gas exchange and fluorescence light response curves on the last fully-expanded leaves of Sunflower (*Helianthus annuus*) and Soybean (*Glycine max*) using the LI-6400XT and 6400-40 (LI-COR, Lincoln, NE, USA)
- The results of the MPF method were cross-checked with an alternate flash train (FT, Figure 1) technique (Markgraf & Berry, 1990; Earl & Ennahli, 2004)
- Φ_{PSII} was calculated as $1 - \frac{Fs}{Fm'}$, where Fs is steady state fluorescence and Fm' was measured via the RF and MPF methods. J, the electron transport rate through PSII, was calculated as $\Phi_{PSII} * f * Q * \alpha_{leaf}$. 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 leaf absorptance, α, was measured with an integrating sphere and spectroradiometer (LI-1800, LI-COR, Lincoln, NE, USA). The values for soybean mesophyll conductance, g_m, were derived using the variable J calculation as

$$g_m = \frac{A}{C_i - \frac{\Gamma^* \cdot (J+8 \cdot (A+R_d))}{J-4 \cdot (A+R_d)}}$$

(Long and Bernacchi, 2003; Pons et al., 2009), where A is net photosynthesis (µmol m^{-2} s^{-1}), C_i is the internal CO_2 concentration (µmol mol^{-1}), Γ^* is the CO_2 compensation point of 46 µmol mol^{-1}, and R_d is the dark respiration rate (µmol m^{-2} s^{-1}).

RF method underestimates Fm'

- The RF method underestimated Fm' by an average of 13% in high-light adapted, field-grown sunflower, resulting in calculated Φ_{PSII} measurements that were 20% lower than those measured via the MPF method (Figure 3).

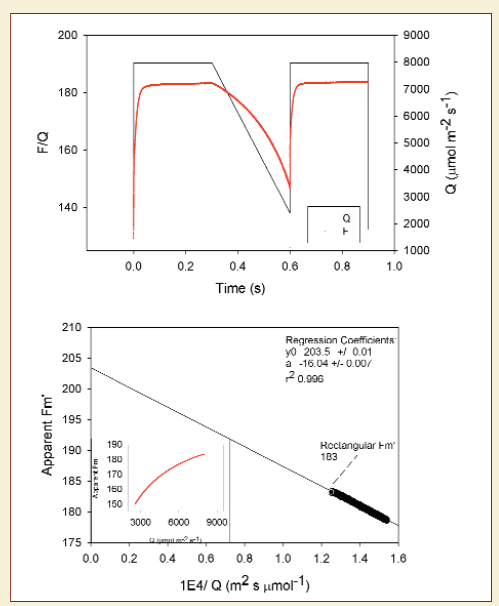

Figure 2. Typical MPF protocol in a field-grown sunflower leaf

Top panel: the typical fluorescence response during a MPF protocol using a 70% (exaggerated, typical ramp is 25%) ramp on a sunflower leaf adapted to 1,800 µmol m^{-2} s^{-1}. **Bottom panel inset:** the fluorescence response to Q during the 70% ramp; **Bottom panel:** the Fm' at infinite flash intensity is found by plotting the fluorescence response during the top 25% of the ramp as a function of 10^4/Q, and extrapolating back to the y-axis.

- The values of Fm' measured by the RF method were underestimated by an average of 8% in high-light adapted, field-grown soybean, resulting in Φ_{PSII} measurements that were 23% lower than those measured via the MPF method (data not shown)

MPF method reliably measures Fm'

- Under easy-to-saturate conditions, a previous study revealed that the MPF method results were not significantly different than those measured by the RF and FT methods (Loriaux et al., 2006)
- In difficult-to-saturate, field-grown and high-light adapted sunflower, Fm' values measured using the MPF method resulted in Φ_{PSII} and J measurements that followed gas exchange measurements (Figure 3). Results in soybean were similar (data not shown).

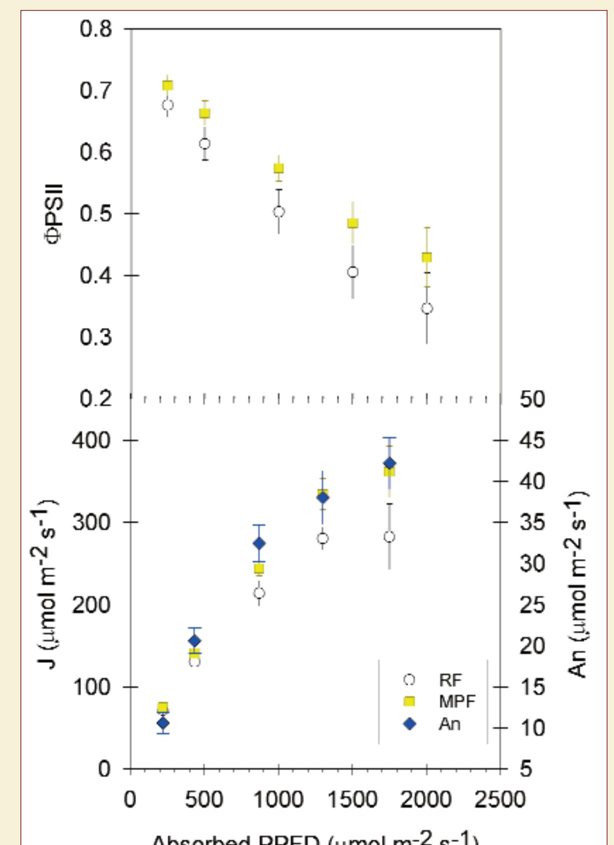

Figure 3. RF and MPF method comparison during light response curves in sunflower

Φ_{PSII} and J were measured using the RF and MPF methods during light response curves measured in field-grown sunflower. Each data point is the mean ± SD of 7 to 9 observations.

- Mesophyll conductance (g_m) for soybean computed using Fm' values from the MPF technique yielded consistent and reasonable values, compared with the highly variable and questionable g_m values (including some negative values) computed using Fm' values from the RF method (Table 1). Results using sunflower data were similar (data not shown).

Table 1. Mesophyll conductance (gm) of soybean calculated using J measured by the RF and MPF methods

Mesophyll conductance values at several incident PPFD levels, calculated using J measured during light response curves on nine soybean leaves using the RF and MPF method. Values are the mean ± standard error. Both methods used a maximum saturating Q of 8,000 µmol m-2 s-1, and the MPF used a 30% drop in intensity during the ramp. Values are means ± SD.

Incident PPFD (µmol m^{-2} s^{-1})	An (µmol m^{-2} s^{-1})	g_s^{CO2} (mol m^{-2} s^{-1})	gm (mol m^{-2} s^{-1} bar^{-1}) RF	gm (mol m^{-2} s^{-1} bar^{-1}) MPF
500	17.5 ± 1.7	0.14 ± 0.035	0.21 ± 0.72	0.43 ± 0.27
1000	22.5 ± 3.3	0.18 ± 0.043	0.72 ± 1.5	0.27 ± 0.044
1500	24.7 ± 4.2	0.20 ± 0.053	-3.6 ± 8.3	0.25 ± 0.042
2000	26.0 ± 5.1	0.23 ± 0.079	-0.30± 0.84	0.28 ± 0.045

Conclusions

- The RF method underestimates Fm' under many conditions, especially in field-grown, high-light adapted plants
- Underestimates in Fm' values not only lead to inaccurate Φ_{PSII} and J values, but also lead to large errors in calculated g_m and C_c values
- The new MPF method can be used to accurately and rapidly estimate the true Fm' under difficult-to-saturate conditions

References

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