Stressed Plants?

Integrated fluorescence and gas exchange measurements allow you to discover more.

- Measure overall plant health using rapid point-measurements or detailed physiological responses
- Integrated, simultaneous measurement of light and dark photosynthetic reactions
- Assessment of light energy allocation in response to stress
- Multiphase Flash™ fluorescence technique for accurately assessing the quantum efficiency of Photosystem II

The balance between light capture and metabolite production in plants is tightly regulated through signal transduction pathways and responses to changes in environmental conditions. Under various environmentally stressful conditions, CO_2 assimilation can be diminished, thereby resulting in excessive absorption of light energy. A large fraction of this excess energy is safely dissipated through an assortment of non-photosynthetic processes, some of which are collectively referred to as non-photochemical quenching (q_N) and are routinely measured as a quenching of chlorophyll *a* fluorescence. q_N is related to pH-dependent limitations in the chloroplast, carotenoid quenching of excitons, phosphate-availability limitations, and heat dissipation, among others.

Integrated measurements of chlorophyll *a* fluorescence and changes in CO_2 concentrations can provide insight into the balance between the capture of light energy, leading to CO_2 assimilation, versus the harmless dissipation of absorbed energy. The light-adapted maximum fluorescence yield (Fm') is used

to calculate many fluorescence parameters, including the rate of electron transport (J) and q_N, which is composed of energy-dependent quenching (q_E), state transitions of the light harvesting antennae (q_T), and a less characterized phenomena thought to involve inhibition of the reaction centers (q_I). To compare the balance of energy allocation to photosynthetic and non-photosynthetic processes, the quantum efficiency of CO_2 uptake (ϕ_{CO2}) and the quantum efficiency of photosystem II electron transport (ϕ_{PSII}) can be compared. Theoretically, ϕ_{CO2} and ϕ_{PSII} are linearly related according to:

$$\Phi_{PSII} = k\Phi_{CO2} + b$$

where the slope *k* is the moles of electrons needed to fix one mole of CO_2 and the intercept *b* is the fraction of electrons going to non-photochemical processes (Genty et al., 1989). As stresses increase, the balance between ϕ_{CO2} and ϕ_{PSII} departs from the theoretical due to a shunting of excess energy to stress response mechanisms. Additionally, J is important in assessing gas-exchange parameters including mesophyll conductance (g_m) and limitations to photosynthesis imposed by triosphosphate utilization (TPU).

Measuring ϕ_{PSII}

In order to measure Φ_{PSII}, it is necessary to estimate the minimum fluorescence yield (Fs) and Fm' under steady-state illumination. Pulse-amplitude modulated (PAM) chlorophyll *a* fluorescence typically uses a very bright pulse of light (Q') with a rectangular wave form (Figure 1), during which the intensity transiently increases (i.e., in this example) from the steady-state intensity of ~2,000 µmol m^{-2} s^{-1} to a maximum of ~7,800 µmol m^{-2} s^{-1}, which is held constant for a very

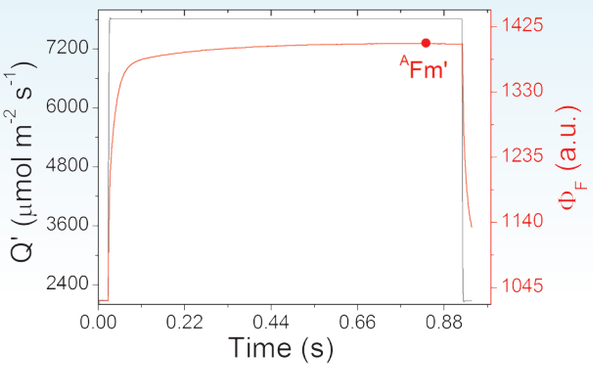

Figure 1

brief duration before returning to the steady-state level (black line). The Q' causes chlorophyll fluorescence (red line) to increase to an apparent maximum (^{A}Fm') value that is typically used to estimate Φ_{PSII}, J, q_N, g_m, and other parameters. The problem is that ^{A}Fm' can underestimate the true Fm', propagating errors in estimation of these derivative parameters. Nonetheless, it has been observed that ^{A}Fm' increases hyperbolically towards an asymptotic maximum (i.e., true Fm') in response to a series of increasing Q' intensities (as reported by Markgraf and Berry, 1990; Earl and Ennahli, 2004). Above a threshold Q' intensity, values of ^{A}Fm' were shown to exhibit linear dependence against the reciprocal of Q' ($(Q')^{-1}$), suggesting that Fm' at infinite irradiance (i.e., true Fm') could be obtained via linear regression and extrapolation to the y-axis. Extrapolated estimates of Fm' (^{E}Fm') have been shown to be invariably better approximations of Fm'.

Multiphase Flash™ Fluorescence

Several flashes, each separated by 1-2 minutes, are required to derive estimates of ^{E}Fm' using the standard extrapolation method, thereby limiting its throughput. Based on the principle of extrapolation, LI-COR® Biosciences has developed a novel technique, herein

referred to as a Multiphase Flash™ fluorescence, to measure the fluorescence data needed to derive ^{E}Fm' in a *single flash event*!

Similar to a traditional Q', a Multiphase Flash™ fluorescence measurement uses a bright pulse of light that rises to a maximum that is held constant for a fixed duration (typically 300 ms) to estimate ^{A}Fm'

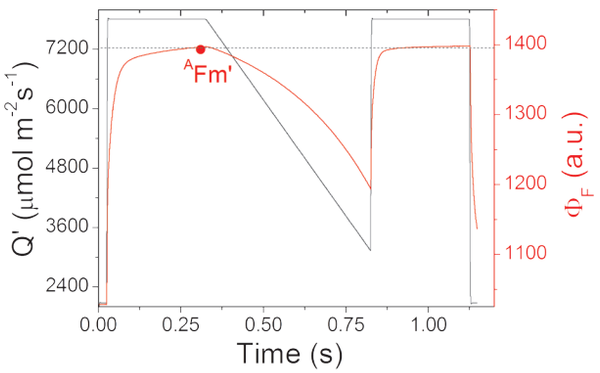

Figure 2

(Figure 2). While continuously measuring chlorophyll fluorescence, the maximum intensity is then linearly attenuated at a user-prescribed rate and amplitude. Chlorophyll fluorescence decreases hyperbolically during this decline in intensity, after which it recovers to the previous value of ^{A}Fm' upon return of the intensity to the initial maximum. It should be noted that deviations between these respective values of ^{A}Fm' can provide diagnostic information as to the induction of potentially harmful auxiliary reactions during the pulse.

Plotting the decreasing fluorescence against $(Q')^{-1}$ (Figure 3), ^{E}Fm' can be determined by simple regression and extrapolation analysis. The resultant value of ^{E}Fm' is shown to be ~10% higher than the corresponding value of ^{A}Fm', both of which were assessed

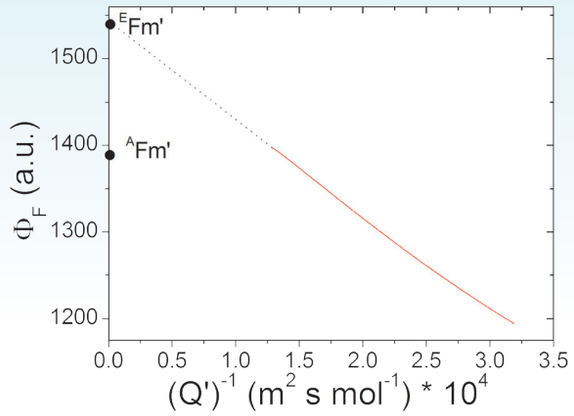

Figure 3

using the same Multiphase Flash™ fluorescence measurement. However, the value of ^{E}Fm' is a superior measurement of Fm' than would have been obtained using a single, standard flash.

Estimates of ^{E}Fm' can be relatively insensitive to increasing Q' intensity. Using Multiphase Flash™ fluorescence, multiple Q' intensities were explored and the resultant values of ^{A}Fm' were compared to the corresponding estimates of ^{E}Fm' (Figure 4). While the values of ^{A}Fm' progressively increased as a function of increasing Q' intensity, those of ^{E}Fm' remained relatively constant at all but the lowest Q' intensity and were between 4-10% higher than the range of values of ^{A}Fm'. These results suggest that more accurate

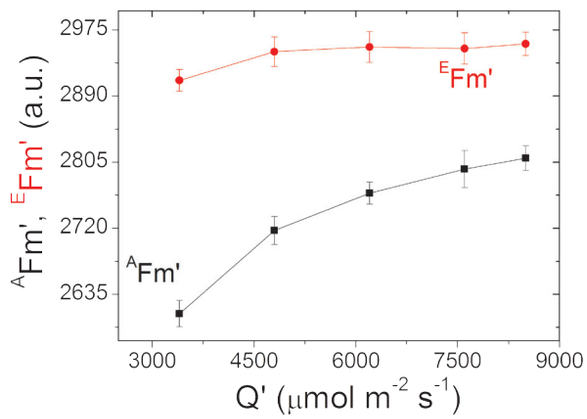

Figure 4

estimates of Fm' (i.e. ^{E}Fm') can be obtained at moderate flash intensities. For plants that are experiencing some form of stress and/or are sensitive to photodamage (i.e., mutants in q_N), using moderate Q' intensities may be advantageous for preventing widespread damage.

The better estimates of Fm' (i.e. ^{E}Fm') obtained using Multiphase Flash™ fluorescence result in closer agreement between the empirical and theoretical rates of J and gross CO_2 assimilation (A_G) for unstressed plants. A series of gas-exchange measurements of A_G and J were made on *Zea mays* (Figure 5). The slope of the relationship between A_G and J based on ^{E}Fm'-derived values of Φ_{PSII} is 4.7 electrons per fixed CO_2 molecule (closed circles), essentially as predicted from theory (Edwards and Baker, 1993). But the slope of the relationship between A_G and estimates of J using

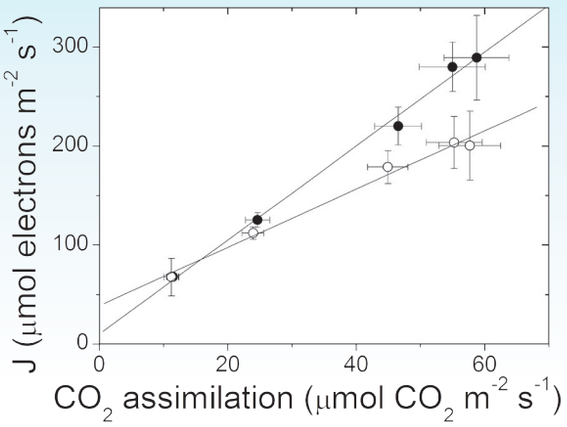

Figure 5

^{A}Fm'-derived values of Φ_{PSII} (i.e. that were obtained using comparably intense standard flashes) is 2.9 electrons per fixed CO_2 molecule (open circles), an unrealistically low value in comparison to the theoretically predicted value. The significant discrepancy is due to underestimation of true Fm' by the values of ^{A}Fm'.

For more details, see the recent publication:

Loriaux, S. D., T. J. Avenson, J. M. Welles, D. K. McDermitt, R. D. Eckles, B. Riensche and B. Genty. 2013. Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity. Plant, Cell & Environment. doi: 10.1111/pce.12115

Citations:

Earl, H. & S. Ennahli. 2004. Estimating photosynthetic electron transport via chlorophyll fluorometry without Photosystem II light saturation. *Photosynthesis Research*. 82:177-186.

Edwards, G. E. & N. R. Baker. 1993. Can CO2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynthesis Research*. 37:89-102.

Genty, B., J-M. Briantais, & N. R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*. 990:87-92

Markgraf, T. & J. Berry. 1990. Measurement of photochemical and non-photochemical quenching: correction for turnover of PS2 during steady-state photosynthesis. In: M. Baltscheffy (ed.), *Current Research in Photosynthesis*, pp. 279-282. Kluwer Academic Publishers, Dordrecht, the Netherlands.

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