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BRIEF COMMUNICATION

Assessment of energy partitioning in PSII complexes using chlorophyll fluorescence: reviewing the different approaches toward the definition of a unified method

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Abstract

Based on the examination and quantitative comparison of the approaches used to assess the energy partitioning in photosystem II, the unified method was proposed to calculate the contribution of the components of nonphotochemical quenching.

Additional key words: chlorophyll fluorescence; energy partitioning; photosystem II.

Nonphotochemical quenching (NPQ) of excitation energy in photosystem II (PSII) has three components characterized by their relaxation kinetics: the fast-relaxing component, also known as the ΔpH-dependent or energy-dependent quenching (q_E), the intermediate component associated with state 2 – state 1 transition (q_T), and the slow component related to PSII photo-damage (q_I). The goal of this paper was to compare and combine several approaches used to assess contribution of q_E, q_T, and q_I to overall PSII energy partitioning. The methodology can be traced back to the work by Cailly *et al.* (1996), where the quantum yield (QY) of NPQ upon illumination was calculated as $\Phi_{NPQ} = \frac{F'}{F_m'} - \frac{F'}{F_m}$. F' and F_m' represent the levels of fluorescence of a light-acclimated leaf with continuous actinic illumination and triggered by a saturating flash, respectively. F_m is the maximum fluorescence level of a dark-acclimated leaf induced by a saturating flash. Φ_{NPQ} is the sum of the QYs of q_E, q_T, and q_I for an illuminated sample (Φ_E, Φ_T, and Φ_I, respectively). A period of dark subsequent a light treatment allows to distinguish between components that relax ($\Phi_E + \Phi_T$) and eventually do not relax (Φ_I) during dark acclimation (Kornyeyev and Holaday 2007):

$$\Phi_E + \Phi_T = \frac{F'}{F_m'} - \frac{F'}{F_m''} \quad (1); \qquad \Phi_I = \frac{F'}{F_m''} - \frac{F'}{F_m} \quad (2)$$

Double prime sign indicates the values of the maximum fluorescence (F_m) measured during dark acclimation following a light treatment (F_m'' can be determined from several minutes to hours of dark relaxation). Ahn *et al.* (2009) applied shorter time of relaxation assuming that 10 min period would allow for relaxation of q_E but not q_T, thereby separating Φ_E and $\Phi_T + \Phi_I$:

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Abbreviations: F_m, F_m', and F_m'' – maximum chlorophyll fluorescence levels of a dark-acclimated leaf, of a light-acclimated leaf, and of a leaf during the dark relaxation after an actinic light treatment, respectively; F_o, F_o', and F_o'' – minimum chlorophyll fluorescence levels of a dark-acclimated leaf, of a light-acclimated leaf, and of a leaf during the dark relaxation after an actinic light treatment, respectively; NPQ – nonphotochemical quenching; PSII – photosystem II; q_E – ΔpH-dependent or energy-dependent quenching; q_I – quenching induced by PSII photodamage; q_T – state transition quenching; QY – quantum yield; $\Phi_{C,F}$ – combined quantum yield of chlorophyll *a* fluorescence and constitutive dissipation; Φ_E – quantum yield of q_E component of NPQ quenching; Φ_I – quantum yield of q_I component of NPQ; Φ_{NPQ} – quantum yield of nonphotochemical quenching; Φ_T – quantum yield of q_T component of NPQ quenching .

$$\Phi_E = \left(\frac{F_m'' - F_m'}{F_m'}\right)\frac{F'}{F_m'} = \frac{F'}{F_m'} - \frac{F'}{F_m''} \qquad (3);$$

$$\Phi_T + \Phi_I = \left(\frac{F_m - F_m''}{F_m}\right)\frac{F'}{F_m''} = \frac{F'}{F_m''} - \frac{F'}{F_m} \qquad (4)$$

Simplifying, Eq. 3 and Eq. 4 look respectively identical to Eq. 1 and Eq. 2. However, the time of dark relaxation prior to measurements of F_m'' (10 min *vs.* several hours), which separates groups of components (Φ_E and $\Phi_T + \Phi_I$ *vs.* $\Phi_E + \Phi_T$ and Φ_I), is different. Φ_T can be estimated by combining two approaches:

$$\Phi_T = \Phi_{E+T} - \Phi_E = \frac{F'}{{F_m''}_{(10\ \mathrm{min})}} - \frac{F'}{{F_m''}_{(3\,\mathrm{h})}}' \qquad (5),$$

where $F_m''_{(10\ \mathrm{min})}$ is F_m'' measured at 10 min of darkness and $F_m''_{(3\,\mathrm{h})}$ is F_m'' measured after 3 h of dark acclimation.

q_E usually relaxes in a very short time (less than 10 min). Thus, Guadagno *et al.* (2010) separated NPQ components by applying the energy partitioning to the well-known triple exponential decay method. It was proposed to detect two points (*x* and *y*) corresponding to the time of the first and the second variation in the slope of NPQ relaxation kinetics measured by following the changes in F_m''. The values of maximal fluorescence at these points (F_{mx}'' and F_{my}'') were used to calculate QYs of all NPQ components:

$$\Phi_E = \left(\frac{F_{mx}'' - F_m'}{F_m'}\right)\frac{F'}{F_{mx}''} = \frac{F'}{F_m'} - \frac{F'}{F_{mx}''} \qquad (6);$$

$$\Phi_T = \left(\frac{F_{my}'' - F_{mx}''}{F_{mx}''}\right)\frac{F'}{F_{my}''} = \frac{F'}{F_{mx}''} - \frac{F'}{F_{my}''} \qquad (7)$$

$$\Phi_I = \left(\frac{F_m - F_{my}''}{F_{my}''}\right)\frac{F'}{F_m} = \frac{F'}{F_{my}''} - \frac{F'}{F_m} \qquad (8)$$

Assuming that F_{mx}'' is an analog of $F_m''_{(10\ \mathrm{min})}$ and F_{my}'' is an analog of $F_m''_{(3\,\mathrm{h})}$, it becomes clear that Eq. 5 is related to Eq. 7 (the other pairs are Eq. 2 and Eq. 8, Eq. 3 and Eq. 6). The sum of $\Phi_E + \Phi_T + \Phi_I$ (Eqs. 6–8) equals to $\frac{F'}{F_m'} - \frac{F'}{F_m}$, which corresponds to formula for Φ_{NPQ}. It has been shown that all approaches examined above are essentially the same from a mathematical point of view. However, the physiological meaning of the parameters is totally related to the length of the dark period chosen for calculations. Therefore, the time intervals used here have to be considered as mere examples and have to be carefully identified for each particular study in preliminary experiments.

Another approach was based on the comparison of levels obtained for quantum yield of photochemistry in PSII with open reaction centers (Kornyeyev and Holaday 2008). This parameter was calculated as $(F_m - F_o)/F_m = F_v/F_m$ for dark-acclimated samples, $(F_m' - F_o')/F_m' = F_v'/F_m'$ for light-acclimated sample, and as $(F_m'' - F_o'')/F_m'' = F_v''/F_m''$ for samples during dark-acclimation previously illuminated (F_o, F_o', and F_o'' are the levels of chlorophyll fluorescence detected for the corresponding situations mentioned above under conditions, when the primary quinone acceptors of all PSII are oxidized). Adapting it to the exponential decay method, we determined the values of F_v''/F_m'' corresponding to the first, $(F_v''/F_m'')_x$, and the second, $(F_v''/F_m'')_y$, variation in the slope of F_v''/F_m'' dark-relaxation kinetic. $(F_v''/F_m'')_y$ is higher than F_v'/F_m', because q_E and q_T are relaxed, when $(F_v''/F_m'')_y$ is measured. By comparison, QYs of PSII with open reaction centers and applying the coefficient F'/F_o' to account for closure of a fraction of PSII reaction centers (Kramer *et al.* 2004) one can obtain the following equation:

$$\Phi_E + \Phi_T = \left(1 - \frac{F_v'/F_m'}{(F_v''/F_m'')_y}\right)\frac{F'}{F_o'} \qquad (9)$$

Φ_E can be estimated by comparing F_v'/F_m', measured when the q_E component is present, and $(F_v''/F_m'')_x$ measured at the time, when q_E is assumed to be fully relaxed:

$$\Phi_E = \left(1 - \frac{F_v'/F_m'}{(F_v''/F_m'')_x}\right)\frac{F'}{F_o'} \qquad (10).$$

Φ_T is calculated by subtracting Φ_E (Eq. 10) from the sum $\Phi_E + \Phi_T$ (Eq. 9). Φ_I for light-acclimated sample under illumination is estimated as following:

$$\Phi_I = \left(1 - \frac{(F_v''/F_m'')_y}{F_v/F_m}\right)\left(\frac{F_v'/F_m'}{(F_v''/F_m'')_y}\right)\frac{F'}{F_o'} \qquad (11)$$

The values of three NPQ components obtained by using the approaches based on measuring F_m (Eqs. 6–8) and F_v/F_m (Eqs. 10–11) are compared in Fig. 1. In this experiment, the garden beet leaves were exposed to light of various intensities for 10 min allowing the induction of the NPQ components at different extents. After the actinic light was turned off, the values of F_o'' and F_m'' were recorded periodically during dark acclimation (*see* Guadagno *et al.* 2010 for details). The strong parallel between the values of the parameters calculated through different methods and the closeness of both slopes to unity suggest that these approaches yield similar results and can be used as alternatives. The deviations from unity can be explained by accidental inaccuracy in F_o'' measurements. The approach based on the changes in F_v/F_m gives more flexibility in designing experimental procedures, because it allows for moving the fiber optic cable of the fluorometer. Small changes in the orientation of the optical cable during a set of measurements may alter the optical path and consequently the absolute levels of F_m'' in a meaningful way. Thus a comparison of the recorded values with previously measured F_m'' would be impracticable. At the same time, the ratio between F_o'' and F_m'' is preserved due to proportional changes in this

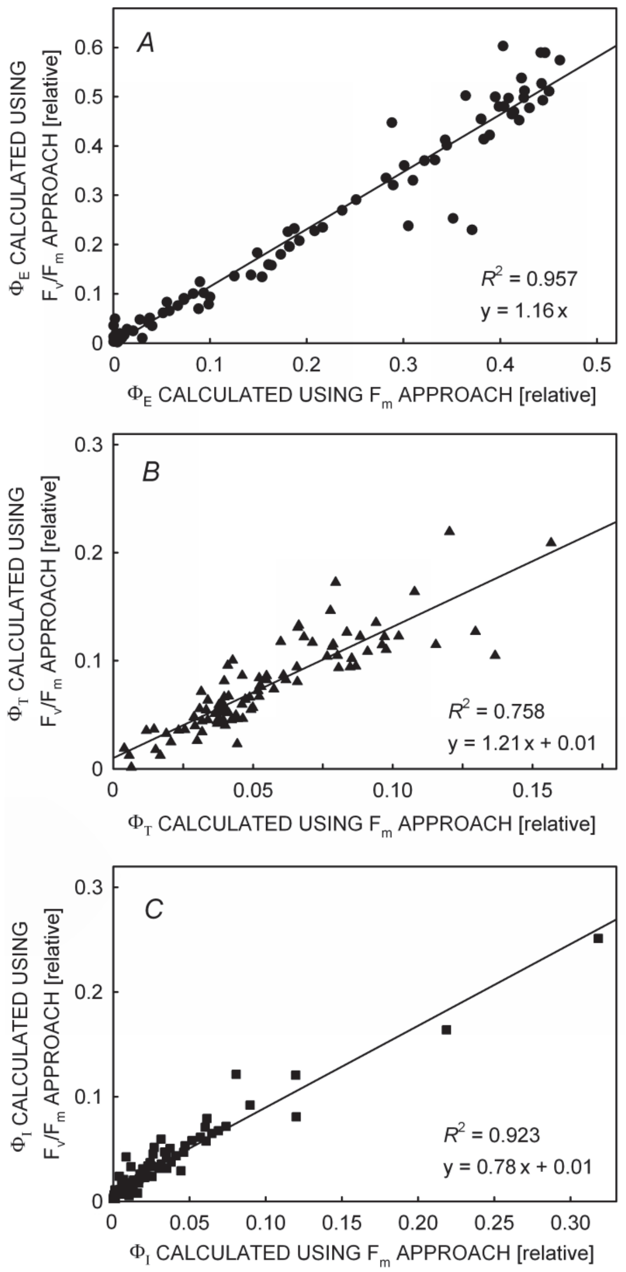

Fig. 1. Relationship between values of quantum yields of three components: q_E (*A*), q_T (*B*), and q_I (*C*) of nonphotochemical quenching obtained by means of two approaches based on the analysis of F_m (F_m'') and F_v/F_m (F_v''/F_m''). The data were obtained at several photon flux densities (80, 180, 450, 750, and 1,200) after the light treatment of *Beta vulgaris* L. leaves for 10 min (63–105 independent measurements for each parameter). See more details in Guadagno *et al.* (2010). In some cases, when $F_m < F_m''$ or $F_v/F_m < F_v''/F_m''$, the negative values were obtained for Φ_E. Those points were not plotted.

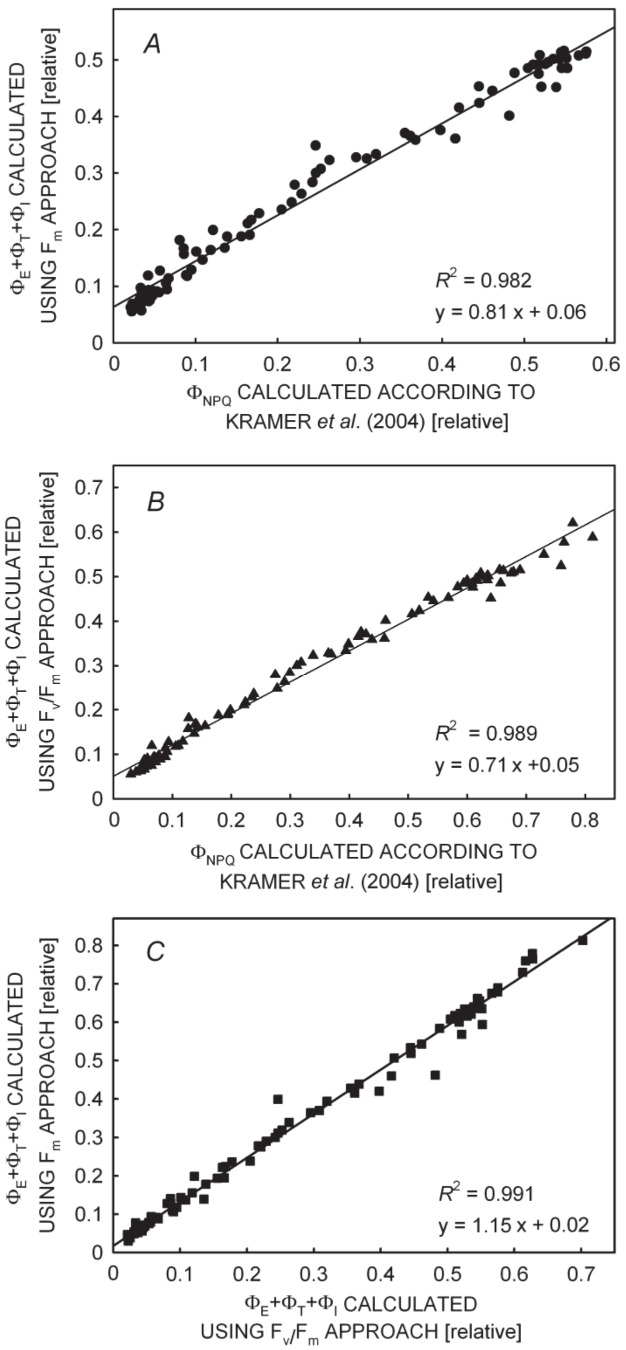

Fig. 2. Relationship between the sum of the quantum yield of three NPQ components ($\Phi_E + \Phi_T + \Phi_I$) obtained using different approaches (F_m; *A*, F_v/F_m, *B*) and the values of quantum yield of light-induced nonphotochemical quenching (Φ_{NPQ}) calculated according to Kramer *et al.* (2004). Panel *C* depicts the correlation between the $\Phi_E + \Phi_T + \Phi_I$ values obtained with F_m and F_v/F_m approaches.

parameters making possible the comparison of F_v''/F_m'' values even after the modification of the optical path ($F_v''/F_m'' = (F_m'' – F_o'')/F_m'' = 1 – F_o''/F_m'$). In some cases, when the changes in F_m (or F_v/F_m) are close to the experimental error, the obtained values of F_m'' (F_v''/F_m'') can be higher than those of F_m (F_v/F_m) leading to negative values of Φ_I calculated according to Eq. 8 (or Eq. 11). A correction can be applied by assuming that $F_{my}''= F_m$ and $(F_v''/F_m'')_y = F_v/F_m$. Note that, in addition to light-induced NPQ, there is a thermal dissipation of excitation energy related to intrinsic properties of PSII (constitutive NPQ) and caused by non-light-induced quenching processes. Unlike light-induced NPQ, constitutive NPQ does not play a role in the defensive regulation of the light energy distribution in photosynthetic apparatus (Hendrickson *et al.* 2004). The contribution of this component is estimated as a combined QY of fluorescence and constitutive dissipation: $\Phi_{C,\,F} = 1 - \Phi_E + \Phi_T + + \Phi_I + \Phi_e$, where Φ_e is QY of electron transport. The analysis based on consideration of the rate constants demonstrates that Φ_E, Φ_T nor Φ_I do not include any portion of constitutive dissipation (Kornyeyev and Holaday 2008, Ahn *et al.* 2009). We also calculated QY of combined light-induced NPQ (Φ_{NPQ}) according to Kramer *et al.* (2004) and, for the first time, we demonstrated an excellent correlation between Φ_{NPQ} and the sum $\Phi_E + \Phi_T + \Phi_I$, which was calculated by using either changes in maximal fluorescence levels (Fig. 2*A*) or quantum yield of PSII photochemistry (Fig. 2*B*). The values of $\Phi_E + \Phi_T + \Phi_I$ obtained with the last two approaches showed an excellent correlation not only to Φ_{NPQ} but also to each other (Fig. 2*C*).

In conclusion, we sorted out two major approaches for detailed characterization of the energy partitioning in PSII complexes. They are based on the changes of either F_m or F_v/F_m during the dark relaxation. The data presented in this paper implied that those approaches could lead to similar results along a wide range of light intensities. However, the calculations based on F_v/F_m seemed to be more practical because of their tolerance to possible fortuitous movements of the optical fiber with consequent positive tradeoffs in case of field or diurnal studies.

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