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LETTER TO THE EDITOR

Inclusion of photoprotective parameters in photosynthesis-measuring systems to improve the interpretation of photosynthesis and productivity

C.C. OGBAGA[*,+] and H.U.R. ATHAR[**]

Department of Biological Sciences, Nile University of Nigeria, Airport Road, Abuja, Nigeria[*]
Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan[**]

While reading the paper by Vredenberg (2018) published in *Photosynthetica* (10 January, 2018), I was inspired to write this letter.

Plant stress is any condition that can negatively affect the morphology, physiology, and biochemistry of plants (Ogbaga *et al.* 2014). Such stress can be biotic or abiotic, induced by pests and non-living factors, such as salinity, drought, *etc*. Plants adopt various mechanisms to respond and adapt to such stresses (Ogbaga *et al*. 2014). Physiologically, stress can be assessed with the use of photosynthesis-measuring systems. Monitoring the degree of stress in crops at early growth stage and timely remedy is essential, otherwise a substantial loss in crop productivity may occur (Ogbaga *et al*. 2018). Conventional methods for crop monitoring are generally time-consuming or involve invasive methods. Similarly, various physiological indicators for selecting stress-tolerant species in breeding programs are also based on invasive techniques.

In the past 40 years, various researchers suggested the use of different chlorophyll (Chl) fluorescence parameters for identifying stress-tolerant cultivars. However, some of them disagree on parameter suitability (a long list of references is given in Desktop Plant Stress Guide Edition 5.0 https://optisci.com/assets/basic_plant_stress_guide_v5.pdf, accessed 25 October, 2018). Little information is available for general users to decide which Chl fluorescence protocol and parameter is the most suitable for screening and selection of stress tolerance. Since 1996, the role of nonphotochemical quenching (NPQ) and cyclic electron transport chain have been significantly studied in stress tolerance (Demmig-Adams and Adams 1996, Niyogi 1999, D'Ambrosio *et al.* 2008, Guadagno *et al.* 2010, Einali *et al.* 2012, Kromdijk *et al.* 2016, Lima Neto *et al.* 2017, Murchie 2017).

Murchie and Niyogi (2011) suggested the manipulation of photoprotective components of NPQ to improve photosynthetic efficiency and crop productivity. Recently, Kromdijk *et al.* (2016) showed an improved crop productivity by manipulating photoprotective mechanism in tobacco. In addition, Goss and Lepetit (2015) demonstrated wide biodiversity in NPQ components in different plant species which suggest that stress-tolerant cultivars can be identified using photoprotective components. In parallel to this development, various researchers have proposed the correct way of calculating NPQ components (Maxwell and Johnson 2000, Guadagno *et al.* 2010, Roháček 2010, Kasajima *et al.* 2015, Tietz *et al.* 2017) that can be used for identifying stress-tolerant cultivars or in assessing efficiency of photoprotective components of NPQ in stress tolerance (Ruban 2016, Giovagnetti *et al*. 2018).

It is well established that NPQ has two main components: *1*) fast relaxation (NPQ_f), *i.e.*, q_E and *2*) slow relaxation (NPQ_s), *i.e.*, q_I. Available published reports suggest that the separation of these components requires detailed understanding of mechanism, technique, and algorithms.

Photosynthesis-measuring systems have been evolved over the years and are currently set up by default to measure Chl *a* fluorescence and gas exchange. General photosynthesis-measuring instruments have default protocols with specific calculated parameters. Often, in order to calculate the parameter of interest, users have to do this by themselves and it requires detailed understanding of protocols and algorithms. Some scientists may help non-specialists calculate it (e-mail/personal communications with Bernardo Duarte, Marine and Environmental Sciences Centre, Faculty of Sciences, University of Lisbon, Portugal). Some researchers provide services on commercial basis (www.fluoromatics.com) or develop supporting software such as *Biolyzer v. 5* compatible with most of fluorometers. However, this company and software was closed in 2015. In view of this information, it is important to include various potential parameters for identification of stress tolerance, such as photoprotective components of NPQ, into photosynthesis-

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[+]Corresponding author; phone: +2349030333632, e-mail: chukwumaogbaga@gmail.com
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Abbreviations: F_m – maximal fluorescence yield of the dark-adapted state; F^o_m – maximum fluorescence at the dark-adapted state; F_m' – maximum fluorescence in the light; F^r_m – F_m in the presence of slowly relaxing quenching; NPQ – nonphotochemical quenching; NPQ_f – fast nonphotochemical quenching; NPQ_s – slow nonphotochemical quenching; q_E – fast component of NPQ; q_I – slow component of NPQ; q_T – state transitions; q_Z – zeaxanthin dependent quenching.

measuring systems. Inclusion of such parameters would help plant breeders or non-specialists in phenotyping using Chl fluorescence (Kromdijk *et al*. 2016, Ruban 2016).

Popular photosynthesis-measuring systems are *Licor 6400/6800* (*Licor*, Nebraska, USA), *CIRAS-1*, *2*, *3* (*PP Systems*, Massachusetts, USA), *Walz* PAM systems (*Heinz Walz*, Effeltrich, Germany), *FluorPen FP 100/110* (*Photon System Instruments*, Drásov, Czech Republic), and recently *MultispeQ v. 2.0* from David Kramer's laboratory (Michigan, USA). None of these machines calculates q_E and q_I on-the-spot. Users are advised to do the calculation at their own convenience. *Walz* PAM systems provide $Y_{(NO)}$ quantum yield of non-regulated heat dissipation, while *MultispeQ v. 2.0* calculates NPQ_t as photoprotective NPQ. The only photosynthesis-measuring system, which measures the parameters, is the fluorescence meter *OSP5* (*Opti-Sciences*, New Hampshire, USA).

Licor, for instance, currently uses a pulse amplitude modulator (PAM) fluorometer for measuring F_0, F_m, F, F_m', and F_0', whilst parameters, such as F_v, F_v/F_m, F_v'/F_m', Φ_{PSII}, q_P, q_N, NPQ, and ETR are calculated (https://www.licor.com/documents/ifuhfcjga0wvh94lkysz, accessed September 9, 2018). These measurements can be performed on both dark- and light-adapted leaves. Since NPQ is calculated in the current version, it should be possible to add the calculation of fast and slow relaxation kinetic parameters using the formulae given by Maxwell and Johnson (2002):
$NPQ_S = (F^o_m - F^r_m)/F^r_m$,
$NPQ_F = (F^o_m/F'_m) - (F^o_m/F^r_m)$,
where F^o_m is the maximum fluorescence at the dark-adapted state, F'_m is the maximum fluorescence in the light, and F^r_m is F_m in the presence of slowly relaxing quenching. This makes measurements more convenient for users interested in distinguishing fast relaxation quenching (q_E) from slow quenching (q_I) which is indicative of photoinhibition. It would be also useful particularly in situations where users must collect massive data in the field.

Currently, NPQ estimates and its associated components (q_E, q_I, q_Z, and q_T) are derived from PAM measurements of Chl fluorescence yield; in order to obtain the maximal yield of fluorescence, leaves have to be fully dark-adapted (F_m) (Tietz *et al*. 2017). This approach implies a prolonged dark acclimation and prevents high-throughput use particularly under field conditions (Tietz *et al.* 2017). It also introduces artefacts when F_m is measured in the presence of photodamaged centres (Tietz *et al*. 2017). Hence, separation of the contributions of NPQ and inclusion in photosynthesis-measuring systems is crucial for plant phenotyping. Thus, the development of new parameters and protocols should be brought into focus in a subsequent new generation of fluorometers.

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