

## REVIEW

## Chlorophyll *a* fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses?#

A. STIRBET<sup>\*,+</sup>, D. LAZÁR<sup>\*\*,+</sup>, J. KROMDIJK<sup>\*\*\*</sup>, and GOVINDJEE<sup>\*\*\*\*</sup>

204 Anne Burras Lane, Newport News, Virginia 23606, USA\*

Department of Biophysics, Center of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 783 71 Olomouc, Czech Republic\*\*

Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA\*\*\*

Department of Biochemistry, Department of Plant Biology, and Center of Biophysics and Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA\*\*\*\*

### Abstract

Chlorophyll (Chl) *a* fluorescence induction (transient), measured by exposing dark-adapted samples to high light, shows a polyphasic rise, which has been the subject of extensive research over several decades. Several Chl fluorescence parameters based on this transient have been defined, the most widely used being the  $F_V [= (F_M - F_0)]/F_M$  ratio as a proxy for the maximum quantum yield of PSII photochemistry. However, considerable additional information may be derived from analysis of the shape of the fluorescence transient. In fact, several performance indices (PIs) have been defined, which are suggested to provide information on the structure and function of PSII, as well as on the efficiencies of specific electron transport reactions in the thylakoid membrane. Further, these PIs have been proposed to quantify plant tolerance to stress, such as by high light, drought, high (or low) temperature, or N-deficiency. This is an interesting idea, since the speed of the Chl *a* fluorescence transient measurement (<1 s) is very suitable for high-throughput phenotyping. In this review, we describe how PIs have been used in the assessment of photosynthetic tolerance to various abiotic stress factors. We synthesize these findings and draw conclusions on the suitability of several PIs in assessing stress responses. Finally, we highlight an alternative method to extract information from fluorescence transients, the *Integrated Biomarker Response*. This method has been developed to define multi-parametric indices in other scientific fields (e.g., ecology), and may be used to combine Chl *a* fluorescence data with other proxies characterizing CO<sub>2</sub> assimilation, or even growth or grain yield, allowing a more holistic assessment of plant performance.

*Additional key words:* JIP-test; Kautsky effect; performance index; tolerance to stress.

Received 13 July 2017, accepted 24 October 2017, published as online-first 10 January 2018.

\*Corresponding authors; e-mails: [sstirbet@gmail.com](mailto:ssstirbet@gmail.com); [lazard@seznam.cz](mailto:lazard@seznam.cz)

*Abbreviations:* ABS – photon flux absorbed by the antenna of PSII units; Area – area above the OJIP transient; CFI – chill factor index; Chl – chlorophyll; CS – cross section; Cyt – cytochrome; DF – driving force; DFI – drought factor index; DI – flux of energy dissipation (through processes other than trapping) in the antenna of PSII units; ET – rate of electron transport from the reduced Q<sub>A</sub> to the intersystem electron acceptors; F<sub>0</sub> – minimum Chl *a* fluorescence; F<sub>d</sub> – ferredoxin; FI – fluorescence induction; F<sub>M</sub> – maximum Chl *a* fluorescence; F<sub>T</sub> – terminal steady state of Chl *a* fluorescence; HSI – heat sensitivity index; I step – Chl *a* fluorescence at ~ 30 ms; IBR – integrated biomarker response; J step – Chl *a* fluorescence at ~ 2 ms; K step – Chl *a* fluorescence at ~ 0.3 ms; M<sub>0</sub> – initial slope (first 0.3 ms) of the O-J fluorescence rise; NPQ – nonphotochemical quenching of the excited states of Chl; OEC – oxygen-evolving complex; P680 – reaction center Chls of PSII; PC – plastocyanin; Phe – pheophytin; P<sub>Si</sub> – photochemical stress index; PI – performance index; PILR – performance index leaf ratio; PQ – plastoquinone; RE – rate of electron transport from the reduced Q<sub>A</sub> to the final electron acceptors of PSI; R<sub>fd</sub> – ratio of fluorescence decrease to steady state fluorescence; ROS – reactive oxygen species; RWC – relative water content; SFI – structure-function index; S<sub>m</sub> – normalized area above the OJIP transient; TR – flux of exciton trapping by active PSII reaction centers leading to Q<sub>A</sub> reduction; ΔV<sub>IP</sub> – relative amplitude of the I-P phase of Chl *a* fluorescence.

*Acknowledgement:* D.L. was supported by a grant # LO1204 (Sustainable Development of Research in the Centre of the Region Haná) from the National Program of Sustainability I, Ministry of Education, Youth and Sports, Czech Republic. J.K. was supported by the Bill and Melinda Gates Foundation (grant OPP1060461). Govindjee was supported by the Department of Plant Biology (James Dalling, Head), and the Department of Biochemistry (Susan Martinis, Head) of the University of Illinois at Urbana-Champaign, IL, USA.

# **Three of the authors (Alexandrina Stirbet, Dušan Lazár and Johannes Kromdijk) pay tribute to their coauthor (Govindjee) for his lifetime work, at his 85<sup>th</sup> birthday (on October 24, 2017).** They write: “Besides his many achievements, Govindjee is well

known in the photosynthesis research community for his passion and excitement in relating chlorophyll (Chl) *a* fluorescence to photosynthesis; this started with his discovery, in 1960, of the two-light effect on Chl *a* fluorescence, and it continues till today.” **Stirbet** wrote: “I met Govindjee in 1995 in Geneva, Switzerland, when he was visiting Reto Strasser’s lab, and we wrote a paper on modeling the Chl *a* fluorescence transient, the OJIP phase (Stirbet *et al.* 1998). In 2010, we restarted our collaboration, and published several reviews, as well as experimental and theoretical papers, still on Chl fluorescence, and I am looking forward to continue our work together”. **Lazár** added: “My first contact with Govindjee dates back to the final stages of my Ph.D. studies, when I submitted a review paper to *Biochimica et Biophysica Acta* on Chl fluorescence induction (Lazár 1999), and Govindjee was one of its reviewers; he had declared his identity. From that time, I know Govindjee as a bright, accurate and enthusiastic scientist, who also supports (and encourages) alternate views [see e.g., my theoretical paper (Lazár 2013) on variable Chl fluorescence originating in PSI, which he had also reviewed].” **Kromdijk** added: “Doing photosynthesis-related research on the Urbana-Champaign Campus of the University of Illinois (USA), it is impossible not to cross paths with Govindjee. I met Govindjee a few years ago after relocating to Urbana-Champaign for my postdoctoral research. Since then, we regularly meet up to drink coffee and talk about life and anything related to photosynthesis. Govindjee has an incredible lifetime of photosynthetic research and experiences to share, and combines this with the passion and enthusiasm of someone who just discovered the topic yesterday.”

## Introduction

Oxygenic photosynthesis involves the conversion of light energy into chemical energy, using two photosystems and carbon fixation enzymes to oxidize water to molecular oxygen and to reduce carbon-dioxide to carbohydrate (see e.g., Blankenship 2014, Govindjee *et al.* 2017). Further, the light energy, absorbed by photosynthetic pigments, has two other fates: loss by internal conversion (as heat) or as light emission (mostly fluorescence). In this review, we explore the use of the latter (see Govindjee *et al.* 1986, Papageorgiou and Govindjee 2004, for a background) to understand abiotic stress on plants. Chlorophyll (Chl) *a* fluorescence is widely used in photosynthesis research, plant physiology, plant phenotyping, remote sensing of plants, and other fields of research that are related to photosynthesis (for reviews, see e.g., Moya and Cerovic 2004, Baker 2008, Meroni *et al.* 2009, Murchie and Lawson 2013, Guanter *et al.* 2014, Kalaji *et al.* 2014a, 2016, 2017a,b; Humplik *et al.* 2015, Mishra *et al.* 2016a).

Chl *a* fluorescence has been used as a highly useful and sensitive signature of photosynthesis (Govindjee 1995, 2004; see also Govindjee *et al.* 1986); it has provided valuable information on the structure and function of the photosynthetic apparatus (Papageorgiou and Govindjee 2004). Measurements of emission and excitation spectra of Chl *a* fluorescence have provided valuable information on various components of the antenna complexes, and on the excitation energy transfer among them (Govindjee *et al.* 1986). Further, fast dynamic changes in Chl *a* fluorescence occur when dark-adapted samples are exposed to light. This induction of Chl *a* fluorescence is also named the Kautsky effect (after Hans Kautsky; see Govindjee 1995, Lazár 1999, Papageorgiou *et al.* 2007, Stirbet and Govindjee 2014) and can be used to extract information about the efficiency of electron transport through PSII (for reviews, see Krause and Weis 1991, Strasser *et al.* 2004, Stirbet and Govindjee 2011, 2012).

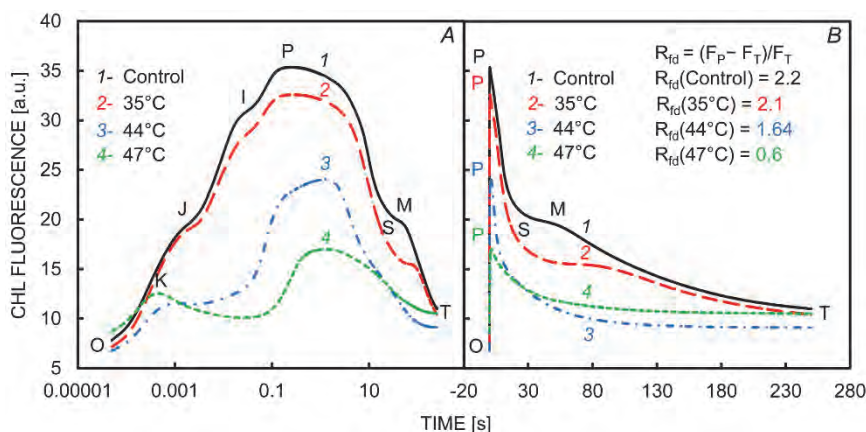


Fig. 1. Chlorophyll *a* fluorescence transients measured in leaves of 10-d-old barley (*Hordeum vulgare* L.) under untreated condition (curve 1, control), and after 5 min at 35°C (curve 2), at 44°C (curve 3), and at 47°C (curve 4). These curves are shown on a logarithmic time scale (A), and on a linear time scale (B); a.u. stands for arbitrary units. The O, K, J, I, P, S, M, and T steps are marked in the figure, where: O is for origin (the minimum fluorescence  $F_0$ ); K, for the fluorescence at 0.3 ms ( $F_K$ ), which has been correlated with the OEC inactivation at high temperatures (Guissé *et al.* 1995); J and I are for the intermediary fluorescence levels at 2 ms and 30 ms ( $F_J$  and  $F_I$ ); and P is for the peak ( $F_P$ ); S stands for a semi-steady state level; M is for a maximum; and T is for the terminal steady state. In (B) we also show the so-called “vitality index”,  $R_{fd} = (F_P - F_T)/F_T$  (Lichtenthaler *et al.* 1986) calculated for each sample. Measurements were made under continuous red (650 nm) light of 2,500  $\mu\text{mol}(\text{photons}) \text{m}^{-2} \text{s}^{-1}$  with a PEA (Plant Efficiency Analyser, Hansatech, UK) instrument.

Table 1. Definition of energy fluxes and fluorescence parameters in the JIP-test (Strasser *et al.* 1999, Tsimilli-Michael and Strasser 2008). These parameters are used to analyze the “fast” (< 1 s) chlorophyll *a* fluorescence transient, the O-J-I-P phase, where O is for origin (the minimum fluorescence  $F_0$ ), J and I for two intermediate levels at 2 ms and 30 ms ( $F_J$  and  $F_I$ ), and P for peak ( $F_P$ , or  $F_M$  when the fluorescence is maximal). Note that PSI, PSII, RC, CS, and  $Q_A$  are for photosystem I, photosystem II, total number of active PSII reaction centers in the measured area, the cross section of PSII, and the first plastoquinone electron acceptor of PSII, respectively.

Technical fluorescence parameters	Meaning
$F_V = F_M - F_0$	Maximum variable fluorescence
$V_t = (F_t - F_0)/F_V$	Relative variable fluorescence
$M_0 = (\Delta V/\Delta t)_0 \approx 4(F_{0.3ms} - F_{0.05ms})/F_V$	Initial slope (in $ms^{-1}$ ) of the O-J fluorescence rise
$Sm = Area/F_V$	Normalized area between the OJIP curve and the line $F = F_M$ , which is a proxy of the number of electron carriers per electron transport chain
Energy fluxes	
ABS	The photon flux absorbed by the antenna of PSII units
TR	The part of ABS trapped by the active PSII units that leads to $Q_A$ reduction
DI	The part of ABS dissipated in PSII antenna in processes other than trapping
ET	The energy flux associated with the electron transport from $Q_A^-$ to the intersystem electron acceptors
RE	The energy flux associated with the electron transport from $Q_A^-$ to the final electron acceptors of PSI
Efficiencies and quantum yields	
$ET_0/TR_0 = \psi E_0 = 1 - V_J$	Efficiency with which a PSII trapped electron is transferred from $Q_A^-$ to PQ
$RE_0/TR_0 = \psi R_0 = 1 - V_I$	Efficiency with which a PSII trapped electron is transferred to final PSI acceptors
$RE_0/ET_0 = \delta R_0 = \psi R_0/\psi E_0$	Efficiency with which an electron from $PQH_2$ is transferred to final PSI acceptors
$TR_0/ABS = \phi P_0 = F_V/F_M$	Maximum quantum yield of primary PSII photochemistry
$ET_0/ABS = \phi E_0 = \phi P_0 \times \psi E_0$	Quantum yield of electron transport from $Q_A^-$ to PQ
$RE_0/ABS = \phi R_0 = \phi P_0 \times \psi R_0$	Quantum yield of electron transport from $Q_A^-$ to final PSI acceptors
$DI_0/ABS = 1 - TR_0/ABS$	Quantum yield of energy dissipation in PSII antenna
Specific energy fluxes (per active PSII)	
$ABS/RC = (M_0/V_J)/\phi P_0$	Apparent antenna size of an active PSII
$TR_0/RC = M_0/V_J$	Maximum trapped exciton flux per active PSII
$ET_0/RC = (M_0/V_J) \times \psi E_0$	The flux of electrons transferred from $Q_A^-$ to PQ per active PSII
$RE_0/RC = (M_0/V_J) \times \psi R_0$	The flux of electrons transferred from $Q_A^-$ to final PSI acceptors per active PSII
$DI_0/RC = ABS/RC - TR_0/RC$	The flux of energy dissipated in processes other than trapping per active PSII
Phenomenological energy fluxes (per CS)	
$ABS/CS_0 \approx F_0$ and $ABS/CS_M \approx F_M$	Absorbed photon flux per excited cross section of PSII
$TR_0/CS = (TR_0/ABS) \times (ABS/CS)$	Maximum trapped exciton flux per cross section of PSII
$ET_0/CS = (ET_0/ABS) \times (ABS/CS)$	The flux of electrons from $Q_A^-$ to PQ per cross section of PSII
$RE_0/CS = (RE_0/ABS) \times (ABS/CS)$	The flux of electrons from $Q_A^-$ to final PSI acceptors per cross section of PSII
Performance indexes	
Performance of PSII and of specific electron transport reactions	
$PI_{ABS} = (RC/ABS) \times [\phi P_0/(1 - \phi P_0)] \times [\psi E_0/(1 - \psi E_0)]$	Performance index on absorption basis
$PI_{ABS,total} = PI_{ABS} \times [\delta R_0/(1 - \delta R_0)]$	Total performance index on absorption basis
$PI_{CS} = PI_{ABS} \times (ABS/CS)$	Performance index on cross section basis
$PI_{CS,total} = PI_{ABS,total} \times (ABS/CS)$	Total performance index on cross section basis
Driving forces	
Performance of PSII and of specific electron transport reactions	
$DF_{ABS} = \log(PI_{ABS})$	Driving force on absorption basis
$DF_{ABS,total} = \log(PI_{ABS,total})$	Total driving force on absorption basis
$DF_{CS} = \log(PI_{CS})$	Driving force on cross section basis
$DF_{CS,total} = \log(PI_{CS,total})$	Total driving force on cross section basis

The rise of Chl *a* fluorescence upon exposure to light is polyphasic (Strasser *et al.* 1995). Plotting a measured fluorescence transient as a function of logarithmic time (Fig. 1A) helps in identifying several key fluorescence levels, sequentially labelled as O-J-I-P-S-M-T [see curves 1 and 2 in Fig. 1, and Govindjee (1995) for the history of this notation]. Here, O is for origin (the dark-adapted minimum fluorescence  $F_0$ ), J and I for two inflections at 2 ms and 30 ms ( $F_J$  and  $F_I$ ), P for peak ( $F_P$ , or  $F_M$  when the fluorescence is maximal, which is usually in the  $\sim 500$ -ms range), S for a semi-steady state, M for a local maximum (which is sometimes missing), and T for the terminal steady-state level (see reviews *e.g.*, by Govindjee and Papageorgiou 1971, Lazár 1999, Papageorgiou *et al.* 2007, Lazár and Schansker 2009, Papageorgiou and Govindjee 2011, Stirbet and Govindjee 2012, Stirbet *et al.* 2014). In addition to these fluorescence levels, sometimes a K level can be observed at 0.3 ms in plants exposed to high temperature (Fig. 1, curves 3 and 4) (Guissé *et al.* 1995).

Many photosynthetic processes influence the kinetics of the fast Chl *a* fluorescence rise from O ( $F_0$ ) to P ( $F_P$  or  $F_M$ ) (reviewed by Lazár 2006, Lazár and Schansker 2009, Schansker *et al.* 2011, 2014; Stirbet and Govindjee 2011, 2012). Considering the case for saturating light, Chl *a* fluorescence intensity increases as the quencher  $Q_A$  becomes more reduced, with all  $Q_A$  reduced at the P level (see Munday and Govindjee 1969a,b). Besides experimental studies, the fast Chl *a* fluorescence rise has been also theoretically simulated (*e.g.*, Stirbet *et al.* 1998, Lazár 2003, 2009, Zhu *et al.* 2005, Hamdani *et al.* 2015).

This O-J-I-P rise measured at saturating light has been widely used to calculate  $(F_M - F_0)/F_M = F_V/F_M$ , a fluorescence parameter that serves as a proxy for the maximum quantum yield of PSII photochemistry (Butler and Kitajima 1975, Govindjee 1995, 2004) and in higher plants, it usually ranges from 0.78 to 0.84 (Björkman and Demmig 1987). However, several other parameters, based on the O-J-I-P transient, have been proposed, especially by Strasser and Strasser (1995) (see also Strasser *et al.* 1999, 2000, 2004, Tsimilli-Michael and Strasser 2008). These proposed parameters (Table 1) are derived by using not only  $F_0$  and  $F_M$ , but also fluorescence levels at J, I, and K.

## Performance indices

We summarize below the derivation of PIs from Chl *a* fluorescence O-J-I-P transients measured under saturating light, which had been proposed by Strasser *et al.* (1999) and Tsimilli-Michael and Strasser (2008). These PIs are calculated with mathematical formulae that capture (and integrate) information contained in 3–4 fluorescence parameters in one number, which is then used to rank different samples according to their PSII and electron transport performance. Less mathematically inclined readers may simply consider the PIs as indices computed based on the aforementioned fluorescence levels and

In this review, we focus on so-called "performance indices" (PIs) proposed by Strasser *et al.* (1999) and Tsimilli-Michael and Strasser (2008). These PIs are proposed to combine information on the performance of PSII and efficiencies of specific electron transport reactions in the thylakoid membrane during the O-J-I-P rise to provide a sensitive tool, *e.g.*, for photosynthetic stress tolerance. This is an interesting idea, since the speed of the measurement ( $<1$  s) is very suitable for high-throughput phenotyping and the obligatory dark-adaptation of the sample reduces the intensity (*i.e.*, power) required to fully saturate photochemical quenching, compared to measurements on light-adapted samples. Both attributes make incorporation of this measurement into high-throughput phenotyping platforms (see *e.g.* Fahlgren *et al.* 2015, Humplik *et al.* 2015) relatively straight forward; however, some issues might arise. For example, the high-time resolution needed for the evaluation of PIs requires high computational demands. This issue does not seem to be insurmountable, since results of PIs measured using Chl fluorescence imaging cameras, which are necessary for the PI-based high-throughput phenotyping, have already been reported (Jedrowski and Brüggeman 2015). Also, the time necessary for dark-adaptation might be different for different plant species and, in any case, it represents a delay that contradicts the requirement for high-throughput. Except for plant stress caused by light treatment, this problem can be overcome by measurements at the end of a period of darkness in the dark/light cycle used during the plant growth. A possible methodology to avoid the dark-adaptation in the high-throughput phenotyping using Chl fluorescence has been suggested, *e.g.*, by Mishra *et al.* (2016b).

We now briefly summarize the derivation of PIs from Chl *a* fluorescence measurements. Subsequently, we will describe how PIs have been used in the assessment of photosynthetic tolerance to various abiotic stress factors. Finally, we synthesize these findings, draw conclusions on the suitability of PIs in assessing stress responses, and discuss alternative ways to derive information from Chl *a* fluorescence transients.

continue reading at the section *Application of performance indices to estimate photosynthetic stress tolerance*, where we review the use of PIs to quantify abiotic stress tolerance. (Also see Table 2 for a list of "indexes" based on the PIs and the JIP-test.)

**The JIP test:** The parameters to calculate PIs have been defined in the so-called JIP-test (Strasser and Strasser 1995), which is based on a proposed theory of "energy fluxes" in photosynthetic biomembranes (Strasser 1978, Strasser *et al.* 2000).

Table 2. List of indices defined, based on the performance index (PI<sub>ABS</sub>) and other parameters of the JIP-test (*see* Table 1; Strasser *et al.* 1999, Tsimilli-Michael and Strasser 2008), and used to evaluate the deleterious effects of particular stress conditions on the performance of PSII activity in plants, which reduce their tolerance to these stressors. Note: PI<sub>ABS</sub> is for the performance index on absorption basis, and equals  $(RC/ABS) \times [\phi P_0/(1 - \phi P_0)] \times [\psi E_0/(1 - \psi E_0)]$ , where RC/ABS is for the density of PSII reaction centers,  $\phi P_0$  is for the maximum quantum yield of PSII photochemistry, and  $\psi E_0$  is for the efficiency with which a PSII trapped electron is transferred from Q<sub>A</sub><sup>-</sup> to PQ; Q<sub>A</sub><sup>-</sup> is for the primary plastoquinone electron acceptor; PQ is for plastoquinone in the PQ pool;  $\phi E_0$  is for the quantum yield of the electron transport from Q<sub>A</sub><sup>-</sup> to PQ, F<sub>M</sub> is for the maximum Chl *a* fluorescence, and F<sub>0</sub> is for the minimum Chl *a* fluorescence. Based on the above definition of PI<sub>ABS</sub>, PSII activity is decreased when PI<sub>ABS</sub> has smaller values.

Index	Definition	Description	Reference
“m”, an index that evaluates the tolerance of plants to high salt	Absolute value ( <i>i.e.</i> , positive) of the slope of the straight line that fits the plot of $\log(\text{PI}_{\text{ABS}}(\text{salt treated}) / \text{PI}_{\text{ABS}}(\text{control}))$ vs. $\log(\phi E_0(\text{salt treated}) / \phi E_0(\text{control}))$	A smaller slope “m” indicates a lower decrease in PSII activity due to high salinity ( <i>i.e.</i> , a higher tolerance)	Misra <i>et al.</i> (2001)
CFI, chill factor index	$\log A + 2 \log B + 4 \log C$ , where A, B, C are average PI <sub>ABS</sub> (chilled) / PI <sub>ABS</sub> (control) during the 1 <sup>st</sup> and 2 <sup>nd</sup> , the 3 <sup>rd</sup> , 4 <sup>th</sup> , and 5 <sup>th</sup> and the 6 <sup>th</sup> and 7 <sup>th</sup> night of chilling	A smaller CFI indicates a higher decrease in PSII activity due to chilling during night ( <i>i.e.</i> , a lower tolerance)	Strauss <i>et al.</i> (2006)
DFI, drought factor index	$\log A + 2 \log B$ , where A and B are average PI <sub>ABS</sub> (unwatered) / PI <sub>ABS</sub> (control) during the 1 <sup>st</sup> and the 2 <sup>nd</sup> week of drought stress	A smaller DFI indicates a higher decrease in PSII activity due to drought ( <i>i.e.</i> , a lower tolerance)	Oukarroum <i>et al.</i> (2007), Boureima <i>et al.</i> (2012)
PILR, performance index leaf ratio	PI <sub>ABS</sub> (leaf 3) / PI <sub>ABS</sub> (leaf 1)	A smaller PILR indicates a higher decrease in PSII activity due to nitrogen deficiency ( <i>i.e.</i> , a lower tolerance)	Živčák <i>et al.</i> (2014)
HRI, heat resistance index	$\log(\text{PI}_{\text{HS}}/\text{PI}_{\text{C}}) + 2\log(\text{PI}_{\text{R}}/\text{PI}_{\text{C}})$ , where PI is the PI <sub>ABS</sub> , while C, HS, and R indicate control, high temperature, and recovery after 24 h	A smaller HRI indicates a higher decrease in PSII activity due to high temperature ( <i>i.e.</i> , a lower tolerance)	Jedmovski and Brüggeman (2015)
HSI, heat sensitivity index	The absolute value of the slope of the straight line that fits the plot of $\log(\text{PI}_{\text{ABS}})$ vs. $V_K = (F_{0.3\text{ms}} - F_0)/(F_M - F_0)$	A smaller HSI indicates a lower decrease in PSII activity due to high temperature ( <i>i.e.</i> , a higher tolerance)	Chen <i>et al.</i> (2016), Oukarroum <i>et al.</i> (2016)
PSi, photochemical stress index	This is calculated based on the algorithm for the Integrated Biomarker Response (IBR; <i>see</i> Appendix) by using 15 fluorescence parameters defined in the JIP-test	A smaller PSi indicates a lower decrease in PSII activity and ET in the thylakoid membrane due to environmental stress ( <i>i.e.</i> , a higher tolerance)	Duarte <i>et al.</i> (2017)

In the JIP test, the following five energy fluxes are considered (Fig. 2):

(a) **ABS** (the absorption flux) – The photon flux absorbed by the antenna of PSII reaction centers;

(b) **TR** (the trapping flux) – The excitation energy flux trapped by open PSII reaction centers, leading to the reduction of Q<sub>A</sub><sup>-</sup> (essentially, PSII photochemistry);

(c) **DI** (the dissipation flux) – The excitation energy flux dissipated in the antenna of PSII units through processes other than trapping (*i.e.*, heat, fluorescence, and excitation energy transfer to PSI);

(d) **ET** – The energy flux associated with electron transport from Q<sub>A</sub><sup>-</sup> to the intersystem electron acceptors;

(e) **RE** – The energy flux associated with the electron transport from Q<sub>A</sub><sup>-</sup> to the final electron acceptors of PSI, which then become reduced.

We note that many studies label these fluxes with the letter “J” (*i.e.*, J<sup>ABS</sup> for ABS, J<sup>TR</sup> for TR, J<sup>DI</sup> for DI, J<sup>ET</sup> for ET, and J<sup>RE</sup> for RE) (*e.g.*, Hendrickson *et al.* 2005, Stauffer 2006, Stirbet and Govindjee 2011). Further, ABS should not be confused with the optical light absorbance or absorptance, which is widely used to describe light attenuation through a sample. However, to keep things simple, we will use the notations that have been used earlier in the photosynthesis literature and in the available software.

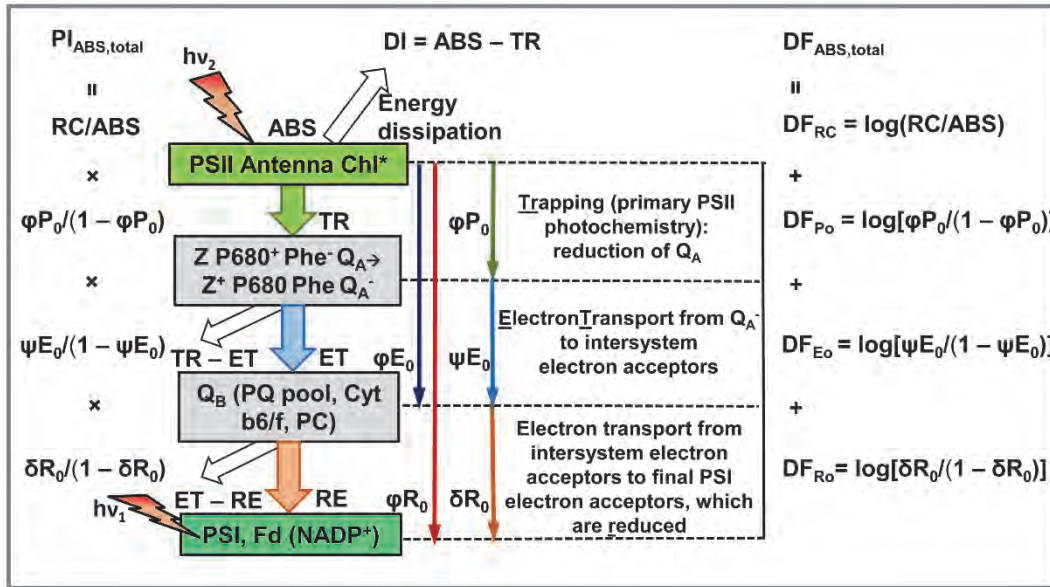


Fig. 2. A diagram showing the relation between several parameters of the proposed JIP-test with the sequential processes taking place during the induction of photosynthesis. ABS is for the photon flux absorbed by the antenna of PSII units; TR is for the part of ABS trapped by PSII reaction centers, leading to the reduction of  $Q_A$ , the primary plastoquinone of PSII; DI is for the part of ABS dissipated in PSII antenna through processes other than exciton trapping; ET is for the energy flux associated with the electron transport from  $Q_A^-$  to intersystem electron acceptors; RE is for the energy flux associated with the electron transport from  $Q_A^-$  to final PSI electron acceptors; RC is for the total number of PSII reaction centers;  $\phi P_0$  ( $= TR_0/ABS$ ),  $\phi E_0$  ( $= ET_0/ABS$ ),  $\phi R_0$  ( $= RE_0/ABS$ ),  $\psi E_0$  ( $= ET_0/TR_0$ ), and  $\delta R_0$  ( $= RE_0/ET_0$ ) are parameters, which are proposed proxies for the ratios of the respective energy fluxes;  $PI_{ABS,total}$  is the total performance index on absorption basis;  $DF_{ABS}$ , or,  $\log(PI_{ABS,total})$  ( $= DF_{RC} + DF_{P_0} + DF_{E_0} + DF_{R_0}$ ) is the total driving force on absorption basis; Z is the secondary donor of PSII (Tyr-161 of D1);  $Q_B$  is the secondary (electron acceptor) plastoquinone of PSII; P680 is the primary electron donor of PSII; Phe is pheophytin, the primary electron acceptor of PSII; Cyt *b<sub>6</sub>/f* is cytochrome *b<sub>6</sub>/f*; PC is plastocyanin; and Fd is ferredoxin. Figure modified from Tsimilli-Michael and Strasser (2008).

Subsequently, an attempt is made to estimate the ratios of these fluxes, based on the fluorescence levels of the O-J-I-P transient, according to the interpretation by Strasser and Strasser (1995) and Tsimilli-Michael and Strasser (2008), as *e.g.*, the quantum yields:

$$\begin{aligned} TR_0/ABS &= 1 - F_0/F_M \\ DI_0/ABS &= F_0/F_M \\ ET_0/ABS &= 1 - F_J/F_M \\ RE_0/ABS &= 1 - F_I/F_M \end{aligned}$$

These equations refer to fluxes at the onset of fluorescence induction (designated by the subscript "0"), whereas the fluorescence levels are determined based on specific times during the transient [*i.e.*,  $F_0$  ( $= F_{0.05ms}$ ),  $F_K$  ( $= F_{0.3ms}$ ),  $F_J$  ( $= F_{2ms}$ ),  $F_I$  ( $= F_{30ms}$ ), and  $F_M$  ( $=$  fluorescence maximum)]. A very important parameter derived in the JIP-test is the ratio  $TR_0/RC$  ( $= M_0/V_J$ ), which represents the maximum trapped exciton flux per active PSII unit; it shows that the initial slope ( $M_0$ ) of the relative variable OJIP fluorescence rise [*i.e.*,  $V_t = (F_t - F_0)/F_V$ ], normalized to  $V_J$  [ $= (F_J - F_0)/F_V$ ], is inversely proportional to the number of PSII reaction centers in the sample (RC). By using  $TR_0/RC$ , the energy fluxes can be expressed as specific energy fluxes (*i.e.*, per active PSII reaction center, *see* Table 1). Furthermore, according to Strasser and

Strasser (1995), several more parameters can be computed from the JIP test which can be grouped as (*see* Table 1):

(1) Yields (*i.e.*, energy fluxes per ABS), and other energy flux ratios: *e.g.*,  $TR_0/ABS$ ,  $DI_0/ABS$ ,  $ET_0/ABS$ ,  $RE_0/ABS$ ,  $ET_0/TR_0$ ,  $RE_0/TR_0$ , and  $RE_0/ET_0$ ;

(2) Specific energy fluxes, divided by the total number of active PSII reaction center(s) (RC): *e.g.*,  $ABS/RC$ ,  $TR_0/RC$ ,  $DI_0/RC$ ,  $ET_0/RC$ ,  $RE_0/RC$  (a notation that may be misleading, as the term RC can be confused with only one PSII reaction center); and

(3) The so-called "phenomenological" fluxes with respect to CS, the cross section of PSII (*i.e.*, the area for photon capture by the light absorbing pigments of PSII antenna; *see* Ley and Mauzerall 1982, Greenbaum *et al.* 1987) are:  $ABS/CS$ ,  $TR_0/CS$ ,  $DI_0/CS$ ,  $ET_0/CS$ ,  $RE_0/CS$ .

All the above parameters have been taken from Strasser and Strasser (1995) and Tsimilli-Michael and Strasser (2008). That is to say, they have been calculated using the fluorescence data from the O-J-I-P transient (*see* formulae in Table 1), but not by using actual energy flux values. Therefore, they are all only proxies of energy ratios, or specific and phenomenological fluxes. [If interested in the theoretical derivation of the parameters used in the JIP-test, *see* reviews by *e.g.*, Strasser *et al.* (1999, 2000, 2004), and Stirbet and Govindjee (2011)].

To construct the PIs described below, Strasser *et al.* (1999) and Tsimilli-Michael and Strasser (2008) had used and combined the following four parameters or ratios (*see* also Table 1 for a summary and list of equivalent nomenclature):

(1) **RC/ABS** or **RC/CS** – the ratio of the total number of active PSII reaction centers (RC) per absorption flux (ABS), or per cross section (CS) of PSII. RC/ABS was estimated by  $(F_V/F_M)/(M_0/V_J)$ , RC/CS<sub>0</sub> by  $F_0 \times (RC/ABS)$ , and RC/CS<sub>M</sub> by  $F_M \times (RC/ABS)$ .

(2) **TR<sub>0</sub>/ABS** (equivalent to  $\phi P_0$ ) – the maximum quantum yield of PSII photochemistry that leads to Q<sub>A</sub> reduction; it was estimated by  $F_V/F_M$ .

(3) **ET<sub>0</sub>/TR<sub>0</sub>** (equivalent to  $\psi E_0$ ) – the efficiency ( $\psi$ ) with which a trapped exciton by PSII reaction center leads to electron transfer (E<sub>0</sub>) from Q<sub>A</sub><sup>-</sup> to PQ, in the PQ pool; it was estimated by  $(F_M - F_J)/F_V$ .

(4) **RE<sub>0</sub>/ET<sub>0</sub>** (equivalent to  $\delta R_0$ ) – the efficiency ( $\delta$ ) of the electron transport (R<sub>0</sub>) from plastoquinol (PQH<sub>2</sub>), in the PQ pool, to the final electron acceptors of PSI (*e.g.*, Fd, NADP<sup>+</sup>, O<sub>2</sub>, thioredoxin); it was estimated by  $(F_M - F_I)/(F_M - F_J)$ .

**Definition of performance indices (PIs):** Based on the above extensive list of parameters, the following PIs were proposed:

**SFI<sub>ABS</sub>** – “structure–function index” on absorption basis: Tsimilli-Michael *et al.* (1998) and Srivastava *et al.* (1999) had defined SFI<sub>ABS</sub> (Eq. 1); it was based on the following parameters: RC/ABS, TR<sub>0</sub>/ABS, and ET<sub>0</sub>/TR<sub>0</sub>, which characterize structural and functional characteristics of PSII (*see* above):

$$SFI_{ABS} = (RC/ABS) \times (TR_0/ABS) \times (ET_0/TR_0) \quad (1)$$

Since a higher amount of active PSII reaction centers (*i.e.*, RC/ABS), a higher quantum yield of PSII photochemistry (*i.e.*, TR<sub>0</sub>/ABS), and a higher efficiency of the electron transport from Q<sub>A</sub><sup>-</sup> to the PQ pool (*i.e.*, ET<sub>0</sub>/TR<sub>0</sub>) would all lead to increased photosynthesis, an increase in SFI<sub>ABS</sub> was suggested to reflect changes that “favor” photosynthesis.

**PI<sub>ABS</sub>** – performance index on absorption basis: PI<sub>ABS</sub> is the most widely used PI, and was proposed by Strasser *et al.* (1999) as a product of RC/ABS and terms in the form  $p_i/(1 - p_i)$ , where the  $p_i$ s ( $i = 1, 2$ ) are TR<sub>0</sub>/ABS and ET<sub>0</sub>/TR<sub>0</sub>:

$$PI_{ABS} = (RC/ABS) \times [(TR_0/ABS)/(1 - TR_0/ABS)] \times [(ET_0/TR_0)/(1 - ET_0/TR_0)] \quad (2)$$

Here, the density of active PSII centers on absorption basis, the RC/ABS, can be also written as  $p/(1 - p)$ , when it is approximated by  $(Chl_{RC}/Chl_{total})/(1 - Chl_{RC}/Chl_{total}) = Chl_{RC}/Chl_{antenna}$ , where:  $Chl_{RC}/Chl_{antenna}$  is the density of active PSII centers per antenna Chl of all PSII, and  $Chl_{total}$  ( $= Chl_{RC} + Chl_{antenna}$ ) is the total Chl concentration in all PSII (*see* Tsimilli-Michael *et al.* 2000).

We note that PI<sub>ABS</sub> (Eq. 2) may be viewed as a ratio

between two SFIs (*see e.g.*, Strasser *et al.* 1999), one that “favors” photosynthesis (SFI<sub>ABS,Po</sub>), and another that does not “favor” photosynthesis (SFI<sub>ABS,No</sub>):

$$SFI_{ABS,Po} = (Chl_{RC}/Chl_{total}) \times \phi P_0 \times \psi E_0 \quad (3)$$

$$SFI_{ABS,No} = (1 - Chl_{RC}/Chl_{total}) \times (1 - \phi P_0) \times (1 - \psi E_0) \quad (4)$$

**PI<sub>ABS,total</sub>** – total performance index on absorption basis: This “newer” index was proposed by Tsimilli-Michael and Strasser (2008) and is calculated by multiplying PI<sub>ABS</sub> with a term in the form of  $p/(1 - p)$ , where “p” is RE<sub>0</sub>/ET<sub>0</sub> (*i.e.*, the proxy of the efficiency with which an electron from PQH<sub>2</sub> is transferred to the final PSI acceptors):

$$PI_{ABS,total} = PI_{ABS} \times [(RE_0/ET_0)/(1 - RE_0/ET_0)] \quad (5)$$

PI<sub>ABS,total</sub> can have positive or negative values, with negative values expressing a “loss” of ability for energy conservation (Yusuf *et al.* 2010). Since PI<sub>ABS,total</sub> is related to the function of the “whole” linear electron transport, whereas PI<sub>ABS</sub> is related only to the electron transport to the PQ pool, it would seem preferable to use only PI<sub>ABS,total</sub>; however, both PI<sub>ABS</sub> and PI<sub>ABS,total</sub> have been used in photosynthesis studies (*see* examples in the section on applications).

Expressing PI<sub>ABS</sub> or PI<sub>ABS,total</sub> per cross-section instead of per reaction center yields four additional PIs, by replacing the RC/ABS in the formula for PI, with RC/CS<sub>0</sub> =  $F_0 \times RC/ABS$  or RC/CS<sub>M</sub> =  $F_M \times RC/ABS$ :

$$PI_{CS_0} = F_0 \times PI_{ABS};$$

$$PI_{CS_0,total} = F_0 \times PI_{ABS,total};$$

$$PI_{CS_M} = F_M \times PI_{ABS};$$

$$PI_{CS_M,total} = F_M \times PI_{ABS,total}.$$

**Definition of driving forces (DFs):** Strasser *et al.* (1999) considered the mathematical formula used to define the PIs as analogous to that used in electrochemistry, with  $p_i$  representing the fraction of the reduced, and  $(1 - p_i)$  the fraction of the oxidized form of a compound. By analogy with the Nernst equation (*see* Nernst 1888), in which  $\log[p_i/(1 - p_i)]$  is used to calculate the reduction potential (the driving force for the corresponding redox reaction), Strasser *et al.* (1999) defined the driving force (DF) to be equal to  $\log(PI)$ , which was suggested to estimate the global driving force of processes evaluated by the corresponding PI. (Note: This DF should not be confused with delayed fluorescence, another name for delayed light emission.) This led to four types of DFs:

$$DF_{ABS} = \log(PI_{ABS});$$

$$DF_{ABS,total} = \log(PI_{ABS,total});$$

$$DF_{CS} = \log(PI_{CS});$$

$DF_{CS,total} = \log(PI_{CS,total})$ , where CS can be CS<sub>0</sub> or CS<sub>M</sub> (*see* above discussion).

In analogy with the Nernst equation, the individual terms  $\log[p_i/(1 - p_i)]$  were considered by Strasser *et al.* (1999) to be analogous to partial potentials for energy conservation for processes related to light absorption by

PSII ( $DF_{RC}$ ), exciton trapping in PSII reaction centers that leads to  $Q_A$  reduction ( $DF_{P_0}$ ), electron transfer from  $Q_A^-$  to the PQ pool ( $DF_{E_0}$ ), and electron transfer from  $PQH_2$  to the final electron acceptors of PSI ( $DF_{R_0}$ ) (Fig. 2): *i.e.*,  $DF_{ABS,total} = DF_{RC} + DF_{P_0} + DF_{E_0} + DF_{R_0}$ .

We note that the analogy between the DFs and the Nernst equation should be seen only as a mathematical formalism since DFs do not represent similar physical

### Application of performance indices to estimate photosynthetic stress tolerance

For growth and survival, plants require extraordinary adaptation potential to the surrounding environment (Bohnert *et al.* 1995, Pareek *et al.* 2010). Abiotic stress factors potentially generate deleterious effects in plants, but at the same time induce complex adaptive mechanisms (*see* reviews by Morales *et al.* 2008, Adams *et al.* 2008, Niinemets 2010, Acosta-Motos *et al.* 2017). Below, we review research using PIs to quantify different abiotic stress effects.

**High light:** Although light is the central driver of photosynthesis,  $CO_2$  assimilation of a typical  $C_3$  species saturates between 30–50 percent of full sunlight. As a result, leaves often receive significantly more light than can be processed by photosynthesis (Adams *et al.* 2008). This can lead to photoinhibition of PSII due to inactivation of PSII reaction centers, damage to the OEC and/or decreased turnover of D1 protein (Hakala *et al.* 2005, Murata *et al.* 2007, Kale *et al.* 2017). To avoid this, several photoprotective processes are induced to help dissipate the excess absorbed energy in a controlled way, such as energy-dependent nonphotochemical quenching (NPQ) of the excited state of chlorophyll (Müller *et al.* 2001) or more sustained NPQ associated with build-up of zeaxanthin (Demmig-Adams 1990, Nilkens *et al.* 2010) *via* reversible de-epoxidation of violaxanthin in the xanthophyll cycle. The kinetics of induction and relaxation of NPQ were recently shown to have a significant impact on biomass productivity of field-grown tobacco (*Nicotiana tabacum*, Kromdijk *et al.* 2016).

Several researchers have measured  $PI_{ABS}$  after prolonged exposure to excessive light. In most cases,  $PI_{ABS}$  was found to be strongly affected by the high-light treatment, much more so than  $F_v/F_m$  (Oukarroum and Strasser 2004, Kalaji *et al.* 2012, Wang *et al.* 2015, and Dinis *et al.* 2016). Oukarroum and Strasser (2004) used  $PI_{ABS}$  in high-light-exposed samples relative to dark-adapted control samples as a way to label ten barley (*Hordeum vulgare* L.) cultivars as sensitive, intermediate, and tolerant to light stress (redrawn in Fig. 3). Unfortunately, no other metrics of high-light stress were determined, which makes it difficult to appreciate the predictive value of this grouping (*see* Table 3).

**Drought:** Drought stress can also have a strong effect on the photosynthetic apparatus. The onset of drought is usually rapidly followed by stomatal closure, reducing

laws, nor real potentials or real driving forces. Therefore, the definition of DF as the sum of partial potentials for energy conservation should not be taken literally. Moreover, since DF is simply another mathematical expression of PI [*i.e.*,  $DF = \log(PI)$ ], it may be considered redundant as an index, since it does not bring any new information beyond that provided by PI.

water loss but also  $CO_2$  influx. This restriction of  $CO_2$  availability ultimately reduces the photosynthetic electron sink, and as a result drought stress is often accompanied by high-light stress (Genty *et al.* 1987, Flexas *et al.* 2004, Morales *et al.* 2008, Chaves *et al.* 2009). Several PIs have been tested to quantify responses to drought stress.  $PI_{ABS}$  was shown to decrease in response to drought stress (Jedrowski *et al.* 2015). An index computed based on  $PI_{ABS}$  measured at two time points during a drought treatment (drought factor index, DFI) was used by Oukarroum *et al.* (2007) to quantify the response of 10 barley varieties to drought. Boureima *et al.* (2012) showed that DFI correlated strongly with an independent leaf temperature based proxy for stomatal conductance in a screen for drought tolerance in 21 sesame (*Sesamum indicum* L.) lines.

Jedrowski *et al.* (2014) studied the effects of drought on  $PI_{ABS,total}$  in several Eurasian and North African genotypes of wild barley. They found that  $PI_{ABS,total}$  was slightly lower in plants exposed to moderate drought than in the controls, when photosynthesis decreases mainly due to stomatal closure. However,  $PI_{ABS,total}$  decreased much more under severe drought, when photosynthesis was also

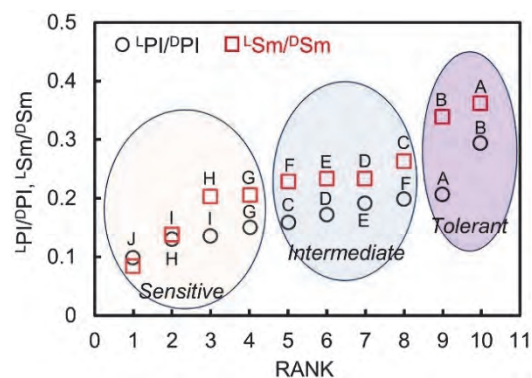


Fig. 3. Ranking of 10 different varieties of barley plants as sensitive, intermediate, and tolerant to high-light stress, according to the values of  $LPI/PI$  and  $LSm/PSm$  ratios; PI is the performance index on absorption basis ( $PI_{ABS}$ ), and  $S_m$  is the normalized Area between the OJIP curve and the horizontal line  $F = F_m$  (*i.e.*,  $S_m = Area/(F_m - F_0)$ , where  $F_m$  and  $F_0$  are the maximum and minimum Chl *a* fluorescence), calculated in samples preilluminated for 5 min with high light (“L”) and in dark-adapted samples (“D”). The letters A to J correspond to specific cultivars. Figure modified after Oukarroum and Strasser (2004).



Table 3. Summary of indices used to quantify response to various stress-factors. Also indicated are whether the responses observed were generic enough to produce a reliable ranking to the stress response, as well as whether the ranking was benchmarked against an independent measurement for the same stress response.

Stress factor	Performance index	Generic response suitable for ranking?	Benchmarked against independent proxy to quantify stress level?
High light	PI <sub>ABS</sub>	Yes	No
Drought	PI <sub>ABS</sub> , DFI	Yes	Leaf temperature proxy for stomatal conductance (Boureima <i>et al.</i> 2012)
	PI <sub>ABS,total</sub>	Not clear, inconsistent results (Jedmowski <i>et al.</i> 2014, Goltsev <i>et al.</i> 2012)	Relative water content (Goltsev <i>et al.</i> 2012)
High temperature	PI <sub>ABS</sub> , log(PI <sub>ABS</sub> ), HSI	Yes	No
	PI <sub>ABS,total</sub>	No, increased in response to high temperature stress (Stefanov <i>et al.</i> 2011)	No
Chilling temperature	PI <sub>ABS</sub> , log(PI <sub>ABS</sub> ), CFI	Not clear. Index showed opposite rank relative to severity of temperature treatment (Ferrante and Maggiore 2007, Baldassare <i>et al.</i> 2011)	CO <sub>2</sub> assimilation and known genotypic sensitivity to chilling (Van Heerden <i>et al.</i> 2003).
			Chlorophyll/carotenoid content (Ferrante and Maggiore (2007).
Freezing temperature	PI <sub>ABS</sub> , PI <sub>ABS,total</sub>	Not clear, inconsistent results.	Respiration rate (Baldassare <i>et al.</i> 2011)
			Survival tests (Rapacz <i>et al.</i> 2015a,b and references therein).
Nutrient limitation	PI <sub>ABS</sub> , PI <sub>ABS,total</sub>	No, responses are distinctly species- and nutrient-specific (Kalaji <i>et al.</i> 2014b)	No
Salinity	PI <sub>ABS</sub>	Yes	No
	PI <sub>ABS,total</sub>	No, inconsistent results (index increased in response to salt stress, Yusuf <i>et al.</i> 2010).	Plant mass
Chromium	PI <sub>ABS</sub>	Yes	Chlorophyll/carotenoid content (Appenroth <i>et al.</i> 2001).
			Respiration and oxygen evolution rates (Appenroth <i>et al.</i> 2001)
Aluminum	PI <sub>ABS,total</sub>	Yes	Chlorophyll content (Jiang <i>et al.</i> 2008) CO <sub>2</sub> assimilation (Jiang <i>et al.</i> 2008)
Ground-level ozone	PI <sub>ABS</sub>	Not clear, index showed opposite rank compared to severity of ozone treatment (Paoletti <i>et al.</i> 2004)	Visual and microscopic damage symptoms (Bussotti <i>et al.</i> 2005).

affected by nonstomatal limitations, such as due to reduced Rubisco content and activity, and increased amount of nonreducing Q<sub>A</sub> PSII centers. In contrast, Goltsev *et al.* (2012) found that PI<sub>ABS,total</sub> in detached bean leaves did not respond to a reduction in relative water content (RWC) from 100–20% and only responded more strongly when leaves were further desiccated from 20% to 0% RWC.

**High temperature:** High leaf temperature increases leaf-to-air vapor pressure deficit, which can exacerbate the effects of drought stress. However, high temperatures in the range of 35–50°C also have more direct negative impact on photosynthesis. Rubisco activase (Feller *et al.* 1998), which is essential for the activation of Rubisco, as well as the OEC (Nash *et al.* 1985) and the CF<sub>0</sub>-CF<sub>1</sub>

ATP-synthase, are all inactivated by high temperature. When the OEC is damaged under high temperature, a new peak appears at ~ 0.3 ms, which has been called the K-peak (Guissé *et al.* 1995, Srivastava *et al.* 1997, Strasser 1997, Lazár *et al.* 1997, 1999). Concurrent with the appearance of the K-peak, F<sub>M</sub> decreases and F<sub>O</sub> increases (Fig. 1). We note that before the K-peak was discovered, the F<sub>O</sub> increase was generally used for screening plants for high temperature sensitivity/resistance (Schreiber and Berry 1977, Nauš *et al.* 1992, Lazár and Ilík 1997). Several studies have looked at the response of PIs to high temperature. Stefanov *et al.* (2011) screened nine bean (*Phaseolus vulgaris* L.) accessions for high-temperature tolerance by exposing plants to 45°C for 2 h followed by two hours of recovery. PI<sub>ABS</sub> decreased in response to the

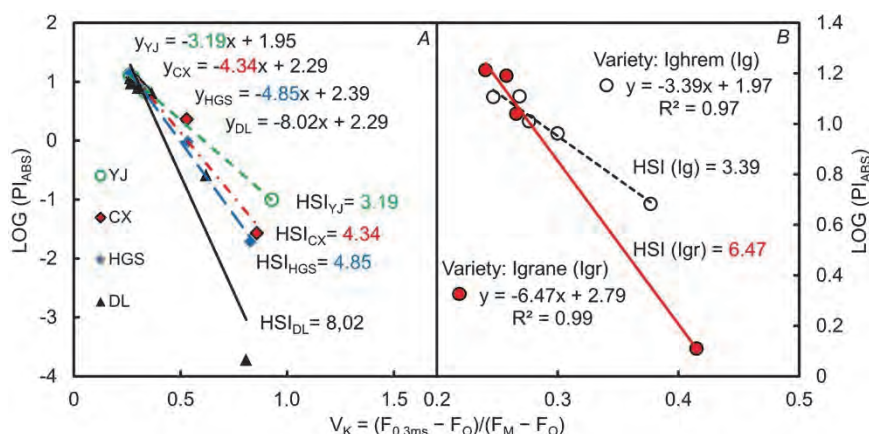


Fig. 4. Analysis of the linear relationship between the logarithm of the performance index,  $\log(\text{PI}_{\text{ABS}})$ , and the relative amplitude of the K-peak,  $V_K = (F_{0.3\text{ms}} - F_0)/(F_M - F_0)$ , calculated from the OJIP fluorescence rise, measured after exposure to high temperature. (A): Croftonweed (*Ageratina adenophora*) plants grown from seeds collected at four different locations in South China: Dali City (DL), Huangguoshu (HGS), Chuxiong City (CX), and Yuanjiang (YJ); and (B): Ighrem (Ig) and Igrane (Igr) varieties of barley (*Hordeum vulgare* L.).  $F_0$  and  $F_M$  are the minimum and maximum Chl *a* fluorescence. The equations of the respective fitted linear trend lines are shown, where “y” is  $\log(\text{PI}_{\text{ABS}})$  and “x” is  $V_K$ . The slope of the fitted linear trend line for each sample is used as heat sensitivity index (HSI), a lower HSI value corresponds to a higher tolerance to high temperature. Graphs modified from Chen *et al.* (2016) and Oukarroum *et al.* (2016).

high-temperature treatment, which is consistent with observations on wheat (*Triticum aestivum*; Mathur *et al.* 2011), barley (*Hordeum vulgare*; Jedmowski and Brüggeman 2015), and sorghum (*Sorghum bicolor* L.; Yan *et al.* 2012). However,  $\text{PI}_{\text{ABS, total}}$  computed from the same data (Stefanov *et al.* 2011) showed an increase in response to high temperature. Chen *et al.* (2016) observed that the response of  $\log(\text{PI}_{\text{ABS}})$  to temperature in croftonweed (*Ageratina adenophora*) was linearly related to the normalized level of the K-peak. The slope of this relationship (named heat sensitivity index, HSI; Fig. 4) was suggested as a measure for plant sensitivity to high temperature stress (Chen *et al.* 2016, Oukarroum *et al.* 2016).

**Low temperature** affects a wide range of physiological processes in plants, including photosynthesis. Low temperature damage to photosynthesis is determined by many factors, such as duration and specific temperature, as well as development stage and interaction with other stress factors (in particular high light). The ability of plants to tolerate low temperatures in the range of 0–15°C is referred to as “chilling tolerance”; on the other hand, “freezing tolerance” is used when there is an enhanced tolerance to freezing temperatures (below 0°C). PIs or  $\log(\text{PIs})$  have been used in several studies on chilling and freezing tolerance (van Heerden *et al.* 2003, 2004a,b; Strauss *et al.* 2006, Ferrante and Maggiore 2007, Liang *et al.* 2007, Rapacz 2007, Rapacz and Woźnicka 2009, Rapacz *et al.* 2015a,b; Adamski *et al.* 2016). Generally, values of  $\text{PI}_{\text{ABS}}$  tend to decrease in response to cold treatment. For example, van Heerden *et al.* (2003) found that six chilling nights at 8°C reduced maximum  $\text{CO}_2$  ( $P_{\text{max}}$ ) assimilation by 32% in a chilling-tolerant, compared to 63% in a chilling-sensitive soybean cultivar, which

matched the change in  $\log(\text{PI}_{\text{ABS}})$  measured in parallel, which also declined more in the chilling-sensitive relative to the chilling-tolerant cultivar.  $F_V/F_M$  was reduced by less than 5% in both cultivars. However, it is not always clear if the decline in  $\text{PI}_{\text{ABS}}$  is a good quantitative predictor of chilling or freezing tolerance. Ferrante and Maggiore (2007) showed that the decrease in  $\text{PI}_{\text{ABS}}$  by low temperature might be associated with the breakdown of chlorophylls and the carotenoids, which was much more pronounced in detached leaves kept at 10°C relative to those kept at 4°C. Further, Baldassare *et al.* (2011) observed a correlation between increased respiration due to low temperature and decreases in  $\text{PI}_{\text{ABS}}$ . We note that the decrease in  $\text{PI}_{\text{ABS}}$  was more pronounced at mild chilling stress (8°C) compared to strong chilling stress (4°C).

$\text{PI}_{\text{ABS}}$  and  $\text{PI}_{\text{ABS, total}}$  have also been observed to decrease in response to freezing temperature (e.g., Rapacz 2007, Fan *et al.* 2015), although rankings based on  $\text{PI}_{\text{ABS}}$  are not always consistent with other measures of freezing tolerance, such as survival tests. Based on these findings it has been suggested by several researchers (see e.g., Rapacz *et al.* 2015a,b) that the use of  $\text{PI}_{\text{ABS}}$  as a proxy for freezing tolerance should be restricted to a specific cold-acclimation pretreatment.

**Nutrient limitation:** Plants require a continuous, balanced supply of several nutrients to achieve optimal growth. Based on the relative elemental abundance in plant dry tissue mass, nutrients can be grouped as macronutrients (greater than 0.1% of dry tissue mass) or as micronutrients (less than 0.01% of dry tissue mass). The macronutrients are: nitrogen, phosphorus, potassium, calcium, sulfur, magnesium, and sodium, whereas micronutrients (or trace minerals) are: boron, chlorine, manganese, iron, zinc,

copper, molybdenum, nickel, and cobalt (Marschner 1995). Design of an optimal nutrient solution was an important theme in early 20<sup>th</sup> century plant science, and the recipe outlined by Hoagland and Arnon (1938) still forms the basis of most nutrient solutions currently used. However, the topic is attracting renewed interest, fueled in particular by the rise of soilless cultivation as an alternative commercial growing system, suitable for urban areas (*e.g.*, van Straten *et al.* 2006).

In a study of a broad range of nutrient deficiencies in maize and tomato (Kalaji *et al.* 2014b), substantial species- and element-specific responses of  $PI_{ABS}$  and  $PI_{ABS, total}$  were observed, with a general decline in both indices due to a decrease in  $F_V/F_M$ . Several other studies looked specifically at nitrogen deficiency. Nitrogen is a strong determinant of plant growth, being both a major component of amino acids (and thus essential for protein synthesis) as well as of chlorophyll. Additionally, up to 50% of total leaf N can be invested in the photosynthetic machinery and  $CO_2$  assimilation, in particular in Rubisco (*e.g.*, Makino 2003). Thus, it is not unreasonable to expect more general responses of PIs to N deficiency. Nitrogen deficiency stimulates redistribution of N from older to younger leaves. To capture this, Živčák *et al.* (2014) defined an index based on several  $PI_{ABS}$  measurements on leaves of different age (performance index leaf ratio), which provided a good indication of total plant nitrogen status. Additionally, Nikiforou and Manetas (2011) found season-dependent and independent nitrogen effects on components of  $PI_{ABS, total}$ . Both of these examples suggest that PIs may be reliably used to assess specific N deficiency.

**Salinity:** Salt stress has a strong impact on agricultural yield, in particular on irrigated land in arid and semiarid regions (Hasegawa *et al.* 2000, Chaves *et al.* 2009). Salt stress affects plants through osmotic stress and ionic stress (Parida and Das 2005, Munns and Tester 2008, Acosta-Motos *et al.* 2017), both of which are likely to affect photosynthesis (Sudhir and Murthy 2004, *see a review on the role of ions in photosynthesis by Kaňa and Govindjee 2016*). Osmotic stress ultimately leads to cell dehydration, whereas ionic stress is caused by excessive accumulation of  $Na^+$  and  $Cl^-$ , reducing uptake of other nutrient ions. As such, the stress symptoms can show similarities with both drought and nutrient limitations, highlighted above.  $CO_2$  availability is affected due to increased diffusion resistance through stomatal pores and into mesophyll cells (Chaves *et al.* 2009), which reduces the photochemical electron sink. On the other hand, PSII activity is reduced by salt stress due to deleterious effects on the Mn cluster of OEC, and PSI activity is also reduced due to the dissociation of plastocyanin/cytochrome  $c_{553}$  (*see e.g.*, Allakhverdiev and Murata 2008). As a result, whole-chain electron transport is also strongly inhibited by salt stress.

Mehta *et al.* (2010) showed that  $PI_{ABS}$  declined in salt-stressed wheat leaves as a result of both ionic and osmotic stress. Furthermore, in a study with mung bean seedlings

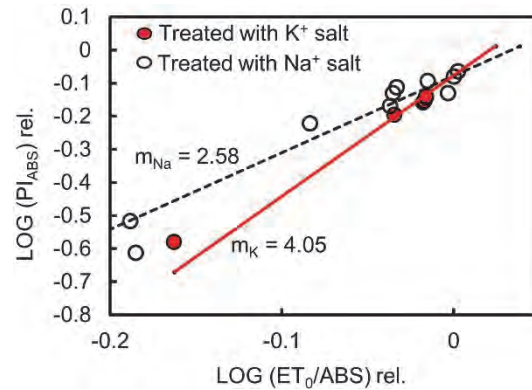


Fig. 5. Analysis of the linear relationship between the logarithm of the relative performance index,  $\log(PI_{ABS})_{rel.}$ , and the logarithm of the relative quantum yield of the electron transport from  $Q_A^-$  to PQ,  $\log(ET_0/ABS)_{rel.}$ , calculated from the OJIP fluorescence induction data, measured on Mung bean seedlings treated with different concentrations of  $K^+$  salt (closed red circles) and  $Na^+$  salt (open circles). The slope “ $m$ ” of the linear trend line fitted for each sample is used as a measure of salt tolerance, a lower “ $m$ ” value corresponding to a higher salt tolerance. Figure modified from Misra *et al.* (2001).

it was shown that the decline of  $PI_{ABS}$  was associated with a decrease in  $(F_M - F_J)/F_V$  (Misra *et al.* 2001). These authors used this relationship to derive a slope between the log-response ratios of  $PI_{ABS}$  and  $(F_M - F_J)/F_V$  to salt (Fig. 5) as an indicator of salt tolerance (Misra *et al.* 2001). Whereas  $PI_{ABS}$  generally declined after exposure to salt, responses of  $PI_{ABS, total}$  to salt appeared less generic.  $PI_{ABS, total}$  decreased significantly in Indian mustard (*Brassica juncea*) plants exposed to salt stress, but showed an increase in transgenic plants overexpressing the  $\gamma$ -tocopherol methyl transferase ( $\gamma$ -TMT) gene (Yusuf *et al.* 2010, Yusuf and Sarin 2007). In the same work, it was also evident that the values of  $PI_{ABS, total}$  did not provide a good indication for growth, since plant mass was not affected by the treatment.

**Pollution:** As it is well known, and discussed above, photosynthetic organisms respond and adapt strongly to the environment they are exposed to. This environment can sometimes be intensely affected by anthropogenic disturbance, in particular in urban areas. Now, we review the use of PIs to quantify stress responses to a number of key contrasting pollutants.

Soils in urban areas are generally contaminated with heavy metals, such as lead, zinc, cadmium, and copper (Alloway 2013), whereas specific industrial use may also lead to other distinct heavy metal contaminants. Although several heavy metal ions, such as those of iron, manganese, magnesium, copper, zinc, cobalt, molybdenum, aluminum, and nickel are essential for plant growth in trace amounts, they are toxic when they exceed a threshold concentration (*see e.g.*, Clijsters *et al.* 1999, Joshi and Mohanty 2004, Nagajyoti *et al.* 2010, Shabnam *et al.* 2017).  $Chl a$

fluorescence measurements have been used for a long time to study toxic effects of heavy metals on photosynthesis, as for example by Bazzaz and Govindjee (1974a) for Cd, and by Bazzaz and Govindjee (1974b) and Wong and Govindjee (1976) for Pb. Here we will highlight a few examples where PIs are used to quantify the effects of Cr (a very common pollutant in the aquatic environment) and Al.  $PI_{ABS}$  decreased in duckweed (*Spirodela polyrrhiza*) exposed to chromate, concurrent with declines in respiration and oxygen evolution rates, as well as decrease in Chl and carotenoid content (Appenroth *et al.* 2001). This decrease in  $PI_{ABS}$  in response to chromate was also observed in the cyanobacterium *Synechocystis sp.* (Pan *et al.* 2009) and seemed to arise mainly from a decrease in  $(F_M - F_J)/F_V$  (similar to that observed by salt stress).  $PI_{ABS, total}$  decreased in Al-treated leaves of grapefruit trees (*Citrus grandis* L.), concomitant with a reduction in Chl content and decline in  $CO_2$  assimilation rate (Jiang *et al.* 2008). Similar to  $PI_{ABS}$  responses to chromate, Al-treated leaves showed a decline of  $(F_M - F_J)/F_V$ , but additionally a K-peak was observed. As previously suggested by Blevins and Lukaszewski (1998), small amount of boron addition alleviated toxic effects of aluminum (Jiang *et al.* 2009), suggesting that aluminum toxicity is at least partially caused by antagonistic effects on boron uptake.

Several studies have used  $PI_{ABS}$  to evaluate city habitats, where soil as well as atmospheric pollution have added significant pressure on tree growth and survival. Hermans *et al.* (2003) used  $PI_{ABS}$  measurements to evaluate positional effects on London plane trees (*Platanus acerifolia* L.) growing in Brussels, on a boulevard with substantial road traffic. Ranking the  $PI_{ABS}$  values relative to the mean of all trees correlated well with other measures of pollution injury and helped the city council to identify areas particularly impacted by pollution. Similarly,  $PI_{ABS}$  measured on trees in Moscow (Russia) corresponded well to differences in exposure to traffic pollution (Volgusheva *et al.* 2011).

The most damaging air pollutant for plant growth is ground-level ozone. Ground-level ozone ( $O_3$ ) forms as a result of a complex interaction between primary pollutants from burning of fossil fuels (nitrogen oxides, volatile organic hydrocarbons, and carbon monoxide) and energy from sunlight. Since these pollutants are able to be transported for a long distance, ozone pollution is not confined to urban areas. Instead, rural areas can also have substantially elevated ground-level ozone, which is harmful for crop yields (Ainsworth *et al.* 2012, McGrath *et al.* 2015).  $PI_{ABS}$  measurements on two trees (*Fraxinus excelsior* L. and *Prunus avium* L.), and a shrub (*Viburnum lantana* L.) species showed increases in response to halving of the ambient ozone concentration (Gravano *et al.* 2004). A negative relationship between  $PI_{ABS}$  and ozone content was also found in several contrasting shrub and tree species, either by filtering ambient air to reduce ozone concentrations (*Fagus sylvatica*, *Populus nigra*, and *Quercus robur*; Bussotti *et al.* 2007) or by looking at ozone

damage in an area naturally subjected to high ozone concentrations (*Fagus sylvatica*, *Acer pseudoplatanus*, *Fraxinus excelsior*, *Viburnum lantana*, and *Ailanthus altissima*; Bussotti *et al.* 2005). In the latter case, a good match was found between  $PI_{ABS}$  and visual or microscopic damage symptoms. Paoletti *et al.* (2004) exposed three Mediterranean evergreen shrubs, laurel (*Laurus nobilis*), a strawberry tree (*Arbutus unedo*), and a phillyrea tree (*Phillyrea latifolia*), to 0, 55, or 110  $nmol\ mol^{-1}$  ozone during 90 days. Here, the negative relationship between ozone exposure and  $PI_{ABS}$  was found for 110  $nmol\ mol^{-1}$  treatment, whereas  $PI_{ABS}$  increased in *L. nobilis* and *A. unedo* at 55  $nmol\ mol^{-1}$  relative to the control treatment.

**Development-specific stresses:** Photosynthetic sensitivity to various stress conditions, and the ability to recover after stress are not only species- and variety-dependent, but they also strongly depend on the developmental stage of the plant.  $PI_{CS_0}$  ( $= F_0 \times PI_{ABS}$ ) was found to gradually increase with leaf development in grapevine (*Vitis vinifera* L., Jiang *et al.* 2006), whereas  $F_V/F_M$  increased only slightly. This gradual increase in  $PI_{CS_0}$  during leaf development originated from increases in  $F_0$  as well as  $(F_M - F_J)/F_V$ . The latter term was also shown to correlate with 820 nm transmittance, measured in parallel as a proxy for PSI quantum yield. Prakash *et al.* (2003) used Chl *a* fluorescence induction to study senescence in cucumber (*Cucumis sativus*) cotyledons.  $PI_{ABS}$  declined from 1.0 to 0.28 to 0.03 in 6, 20, and 27-d-old cotyledons; as a result of a gradual decrease of all component fluorescence parameters that define  $PI_{ABS}$ . Liu *et al.* (2006) examined the damage to PSII during senescence in *Spirodela polyrrhiza* explants under long-day conditions, and its prevention by 6-benzyladenine (a synthetic cytokinin, a class of plant hormones involved in plant growth and development; D'Agostino and Kieber 1999). After 8 days of cultivation under long-day conditions,  $PI_{ABS}$  approached zero, the J and I steps of the OJIP transient disappeared, and the  $F_K$  level was very close to that of  $F_M$ , similar to results by Prakash *et al.* (2003) at advanced stages of senescence.

Duarte *et al.* (2017) studied several seagrass *Zostera noltii* samples, which were in different developmental stages, growing at several sites on the coast of Portugal. Here, response to natural environmental stress factors was evaluated by calculating a novel index, called the photochemical stress index (PSi), which had been defined based on 15 different parameters of the JIP-test. These were considered as biomarkers (*i.e.*, measurable indicators of the photochemical stress). We emphasize that the PSi is very different from the PIs and the DFs defined above. Instead, the PSi was calculated using a general algorithm called Integrated Biomarker Response (IBR) (Beliaeff and Burgeot 2002; for details, see the Appendix) which has previously been used in ecological studies of environmental stress (see *e.g.*, Broeg and Lehtonen 2006, Ferreira *et al.* 2015a,b). Duarte *et al.* (2017) found that PSi values

derived with the IBR protocol decreased substantially with development, from approximately 0.9 at stage 1 to zero at stage 5.

**Concluding remarks:** It is imperative to remember that PIs only evaluate the performance of relatively fast (less than one second) photosynthetic processes in dark-adapted samples. As a result, the predictive value for processes related to CO<sub>2</sub> assimilation, which need longer time to be fully activated, will always remain limited. However, several researchers have assumed that PIs can also provide an assessment of whole-plant “performance”, including CO<sub>2</sub> assimilation, and even growth and grain yield. Although in some cases the PI values were shown to correlate with data related to CO<sub>2</sub> assimilation or even growth (*e.g.*, Strasser *et al.* 2007, Zubek *et al.* 2009), several other results confirm that PIs cannot reliably evaluate these processes (*e.g.*, Yusuf *et al.* 2010, Jedmowski *et al.* 2015). When Chl *a* fluorescence induction is evaluated after exposure to light on a longer time scale (minutes – hours), parameters such as  $F_q'/F_M'$  (also known as  $\Phi_{PSII}$  or Genty’s parameter, Genty *et al.* 1989; *see footnote*<sup>1</sup>) may indeed predict CO<sub>2</sub> assimilation when photorespiration is low (*see e.g.*, Edwards and Baker 1993, Toepel *et al.* 2004). Therefore, results of gas-exchange measurements, or data from Chl FI measured on a longer time scale, should be included if the aim is to analyze the complete time scale of photosynthesis and beyond. This suggestion is in agreement with Kalaji *et al.* (2017b) who concluded that the best way to properly evaluate the performance of plants is to combine many parameters at once. It seems, however, unlikely that growth and yield can ever be reliably predicted from a photosynthesis-based proxy, considering the multitude of processes controlling these complex traits.

In this review, we have discussed several performance indices (PIs) and their applications for the evaluation of stress tolerance of plants to different types of stressors. It is obvious from these results that the O-J-I-P transient contains useful information beyond that from  $F_v/F_M$ , which is most widely used for the evaluation of stress response in plants (*e.g.*, Lazár and Nauš 1998, Kouřil *et al.* 2003, Frolec *et al.* 2010). Including this information has potential to get a more sensitive assessment of stress responses (summarized in Table 3). Whereas the discussed PIs are derived according to the interpretation proposed by Reto J. Strasser and his coworkers, we emphasize that

acceptance of this framework is not required to make use of the information encoded in fluorescence transients. Instead, many researchers have taken a more empirical approach to the use of these PIs, which does not require any interpretation as long as an independent alternative proxy for the stress response is measured in parallel. Indeed, several indices have been customized to better suit the stress factor under evaluation (Table 2), *see e.g.*: (1) Misra *et al.* (2001), for salt stress; (2) Oukarroum and Strasser (2004), for light stress; (3) Strauss *et al.* (2006), for chilling stress at night; (4) Oukarroum *et al.* (2007), for drought stress; (5) Živčák *et al.* (2014), for N-deficiency; (6) Jedmovski *et al.* (2014) and Chen *et al.* (2016), for high temperature stress; and (7) Duarte *et al.* (2017), for natural environmental stress.

These new indices were shown to be more sensitive to differences in the specific stress tolerance between samples than the original PIs. The so-called photochemical stress index (PSi), as used by Duarte *et al.* (2017), merits special attention. Although it still extracts information from fluorescence transients using the so-called JIP test, it has been calculated with a general algorithm designed by Beliaeff and Burgeot (2002), called the **Integrated Biomarker Response (IBR)**. Since the IBR algorithm represents a simple, practical and accommodating method to create well-standardized multi-parametric indexes that can be defined for various applications; we have presented the general steps necessary for its calculation in the Appendix (*see below*). Additionally, we propose that an even more radical approach could be obtained by adopting a fully empirical, unbiased and non-interpretive strategy from the field of hyperspectral imaging (Serbin *et al.* 2012) to obtain the most predictive information from fluorescence transients for the trait of interest. In this method, partial least squares regression analysis was used to derive maximum RuBP carboxylation and regeneration rates from hyperspectral leaf reflectance samples. It seems that this approach could easily be replicated on fluorescence transients to extract the most predictive information for several of the quantitative traits, using ‘training sets’ where both fluorescence transient and trait of interest are determined in parallel. Using these alternative approaches, we anticipate that the information encoded in fluorescence transients, and eventually also from different type of data, can be most efficiently employed in high-throughput screens for stress tolerance.

<sup>1</sup> The so-called Genty’s parameter, named after Bernard Genty, equals  $F_q'/F_M' = (F_M' - F_t)/F_M'$ , where  $F_M'$  is the maximum Chl fluorescence reached during a saturating pulse applied for  $\sim 1$  s at steady state under illumination with actinic light (the light-adapted state), and  $F_t$  is the fluorescence at steady state (subscript “t” is for the time it was measured, just before the measurement of  $F_M'$ ). The above equation reflects the quantum yield of PSII photochemistry for the light-adapted state, and it correlates with the quantum yield of CO<sub>2</sub> assimilation (Genty *et al.* 1989, *see also* Lazár 2015).

## Appendix: Calculation of the Integrated Biomarker Response (IBR) index

We describe below the successive data-processing steps for the calculation of the IBR index (Beliaeff and Burgeot 2002, Broeg and Lehtonen 2006, Ferreira *et al.* 2015a,b). These calculations are referred to biomarkers (*i.e.*, measurable indicators of some biological state or condition) measured at different locations (stations), but they can also be related to surveys made at different periods of time, or to various types of samples, depending on the comparisons needed for one's study.

The steps involved for each biomarker are:

**Step 1.** Calculation of the mean values ( $X$ ) of the biomarkers, and their standard deviations ( $s$ ) corresponding to each station (or type of sample).

**Step 2.** Standardization of the data from each station, which allows their direct visual comparison:  $Y = (X - m)/s$ , where  $Y$  is the standardized value of the biomarker;  $X$  and  $s$  are as defined in Step 1, and  $m$  is the mean value of the biomarker calculated for all stations (or all types of samples).

**Step 3.** Calculation of a quantity  $Z$  as:  $Z = -Y$  or  $Z = +Y$ , if the biological effect on the marker corresponds to an inhibition or a stimulation.

**Step 4.** Calculation of the score ( $S$ ) for each biomarker, where  $S \geq 0$ ; this is done by adding to  $Z$  the absolute (*i.e.*, positive) value of the minimum of the standardized data (*i.e.*, the  $Y$  values calculated at Step 2) calculated for all stations (or types of samples), which is labeled  $|\text{Min}|$ :  $S = Z + |\text{Min}|$ . This leads to the lowest value in the set to be zero.

**Step 5.** The "scores" calculated for all the biomarkers,  $S_i$ , are then represented graphically using a star plot (known also as spider, web, or a radar plot), the star plot radius coordinate representing the score of a given biomarker at a given station.

**Step 6.** Finally, the IBR is calculated from the star plot as:  $\text{IBR} = \sum_{i=1}^n A_i$ , where:  $n$  is the number of biomarkers, and  $A_i$  corresponds to the area of the triangle formed by two consecutive scores (radius coordinates),  $S_i$  and  $S_{i+1}$ , and the center of the star plot. The area  $A_i$  is calculated as:  $A_i = (S_i/2)\sin\beta(S_i\cos\beta + S_{i+1}\sin\beta)$ , with  $\beta = \text{Arctan} [(S_{i+1}\sin\alpha)/(S_i - S_{i+1}\cos\alpha)]$ ,  $\alpha = 2\pi/n$ , and  $S_{n+1} = S_1$  (*see* an example below). If two consecutive scores are zero, the respective  $A_i$  is set to 0.

The IBR, thus calculated, represents the average of different arrangements of the biomarkers in the star plot. The formula for IBR is much simpler when only four biomarkers are used (*i.e.*, when  $n = 4$  and  $\alpha = \pi/2$ ), with:  $A_i = (S_i \cdot S_{i+1}/2)$ ,  $\text{IBR} = (S_1 \cdot S_2 + S_2 \cdot S_3 + S_3 \cdot S_4 + S_4 \cdot S_1)/2$ . We note that, since IBR is directly dependent on the number ( $n$ ) of biomarkers in the set,  $\text{IBR}/n$  must be used for comparing different samples (Broeg and Lehtonen 2006, Ferreira *et al.* 2015a,b).

## References

- Acosta-Motos J.R., Ortuño M.F., Bernal-Vicente A. *et al.*: Responses to salt stress: Adaptive mechanisms. – *Agronomy* **7**: 18, 2017.
- Adams III W.W., Zarter C.R., Mueh K.E. *et al.*: Energy dissipation and photoinhibition: A continuum of photoprotection. – In: Demmig-Adams B., Adams III W.W., Mattoo A.K. (ed.): *Photoprotection, Photoinhibition, Gene Regulation, and Environment*. Pp. 49-64. Springer Science+Business Media B.V., Dordrecht 2008.
- Adamski J.M., Cargnelutti D., Sperotto R.A. *et al.*: Identification and physiological characterization of two sister lines of indica rice (*Oryza sativa* L.) with contrasting levels of cold tolerance. – *Can. J. Plant Sci.* **96**: 197-214, 2016.
- Ainsworth E.A., Yendrek C.R., Stitch S. *et al.*: The effects of ozone on net primary productivity and implications for climate change. – *Annu. Rev. Plant Biol.* **63**: 637-661, 2012.
- Allakhverdiev S.I., Murata N.: Salt stress inhibits photosystem II and I in cyanobacteria. – *Photosynth. Res.* **98**: 529-539, 2008.
- Alloway B.J.: Sources of heavy metals and metalloids in soils. – In: Alloway B. (ed.): *Heavy Metals in Soils*. Environmental Pollution, Vol. 22. Springer, Dordrecht 2013.
- Appenroth K.J., Stöckel J., Srivastava A. *et al.*: Multiple effects of chromate on the photosynthetic apparatus of *Spirodela polyrhiza* as probed by OJIP chlorophyll *a* fluorescence measurements. – *Environ. Pollut.* **115**: 49-64, 2001.
- Baker N.R.: Chlorophyll fluorescence: A probe of photosynthesis *in vivo*. – *Annu. Rev. Plant Biol.* **59**: 89-113, 2008.
- Baldassarre V., Cabassi G., Ferrante A.: Use of chlorophyll *a* fluorescence for evaluating the quality of leafy vegetables. – *Aust. J. Crop Sci.* **5**: 735-741, 2011.
- Bazzaz M.B., Govindjee: Effects of cadmium nitrate on spectral characteristics and light reactions of chloroplasts. – *Environ. Lett.* **6**: 1-12, 1974a.
- Bazzaz M.B., Govindjee: Effects of lead chloride on chloroplast reactions. – *Environ. Lett.* **6**: 175-191, 1974b.
- Beliaeff B., Burgeot T.: Integrated biomarker response: a useful tool for ecological risk assessment. – *Environ. Toxicol. Chem.* **21**: 1316-1322, 2002.
- Björkman O., Demmig B.: Photon yield of O<sub>2</sub> evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. – *Planta* **170**: 489-504, 1987.
- Blankenship R.E.: *Molecular Mechanisms of Photosynthesis*, 2<sup>nd</sup> ed. Pp. 312. Blackwell-John Wiley, Oxford 2014.
- Blevins D.G., Lukaszewski K.M.: Boron in plant structure and function. – *Annu. Rev. Plant Phys.* **49**: 481-500, 1998.
- Bohnert H.J., Nelson D.E., Jensen R.G.: Adaptations to environmental stresses. – *Plant Cell* **7**: 1099-1111, 1995.
- Boureima S., Oukarroum A., Diouf M. *et al.*: Screening for drought tolerance in mutant germplasm of sesame (*Sesamum indicum*) probing by chlorophyll *a* fluorescence. – *Environ. Exp. Bot.* **81**: 37-43, 2012.
- Broeg K., Lehtonen K.K.: Indices for the assessment of environmental pollution of the Baltic Sea coasts: Integrated assessment of a multi-biomarker approach. – *Mar. Pollut. Bull.*

- 53: 508-522, 2006.
- Bussotti F., Agati G., Desotgiu R. *et al.*: Ozone foliar symptoms in woody plants assessed with ultrastructural and fluorescence analysis. – *New Phytol.* **166**: 941-955, 2005.
- Bussotti F., Strasser R.J., Schaub M.: Photosynthetic behavior of woody species under high ozone exposure probed with the JIP-test: A review. – *Environ. Pollut.* **147**: 430-437, 2007.
- Butler W.L., Kitajima M.: Fluorescence quenching in photosystem II of chloroplasts. – *Biochim. Biophys. Acta* **376**: 116-125, 1975.
- Chaves M.M., Flexas J., Pinheiro C.: Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. – *Ann. Bot.-London* **103**: 551-560, 2009.
- Chen S., Yang J., Zhang M. *et al.*: Classification and characteristics of heat tolerance in *Ageratina adenophora* populations using fast chlorophyll *a* fluorescence rise O-J-I-P. – *Environ. Exp. Bot.* **122**: 126-140, 2016.
- Clijsters H., Cuypers A., Vangronsveld J.: Physiological responses to heavy metals in higher plants; Defence against oxidative stress. – *Z. Naturforsch.* **54c**: 720-734, 1999.
- D'Agostino I.B., Kieber J.J.: Molecular mechanisms of cytokinin action. – *Curr. Opin. Plant Biol.* **2**: 359-364, 1999.
- Demmig-Adams B., Adams W.W., Heber U. *et al.*: Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. – *Plant Physiol.* **92**: 293-301, 1990.
- Denin L.-T., Ferreira H., Pinto G. *et al.*: Kaolin-based, foliar reflective film protects photosystem II structure and function in grapevine leaves exposed to heat and high solar radiation. – *Photosynthetica* **54**: 47-55, 2016.
- Duarte B., Pedro S., Marques J.C. *et al.*: *Zostera noltii* development probing using chlorophyll *a* transient analysis (JIP-test) under field conditions: Integrating physiological insights into a photochemical stress index. – *Ecol. Indic.* **76**: 219-229, 2017.
- Edwards G.E., Baker N.R.: Can CO<sub>2</sub> assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? – *Photosynth. Res.* **37**: 89-102, 1993.
- Fahlgren N., Gehan M.A., Baxter I.: Lights, camera, action: high-throughput plant phenotyping is ready for a close up. – *Curr. Opin. Plant Biol.* **24**: 93-99, 2015.
- Fan J., Hu Z., Xie Y. *et al.*: Alleviation of cold damage to photosystem II and metabolisms by melatonin in Bermudagrass. – *Front. Plant Sci.* **6**: 925, 2015.
- Feller U., Crafts-Brandner S.J., Salvucci M.E.: Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. – *Plant Physiol.* **116**: 539-546, 1998.
- Ferrante A., Maggiore T.: Chlorophyll *a* fluorescence measurements to evaluate storage time and temperature of *Valeriana* leafy vegetables. – *Postharvest Biol. Tec.* **45**: 73-80, 2007.
- Ferreira N.G.C., Cardoso D.N., Morgado R. *et al.*: Long-term exposure of the isopod *Porcellionides pruinosus* to nickel: costs in the energy budget and detoxification enzymes. – *Chemosphere* **135**: 354-362, 2015b.
- Ferreira N.G.C., Morgado R., Santos M.J.G. *et al.*: Biomarkers and energy reserves in the isopod *Porcellionides pruinosus*: The effects of long-term exposure to dimethoate. – *Sci. Total Environ.* **502**: 91-102, 2015a.
- Flexas J., Bota J., Loreto F. *et al.*: Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. – *Plant Biol.* **6**: 269-279, 2004.
- Frolec J., Řebíček J., Lazár D. *et al.*: Impact of two different types of heat stress on chloroplast movement and fluorescence signal of tobacco leaves. – *Plant Cell Rep.* **29**: 705-714, 2010.
- Genty B., Briantais J.-M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – *Biochim. Biophys. Acta* **990**: 87-92, 1989.
- Genty B., Briantais J.-M., Da Silva J.B.V.: Effect of drought on primary photosynthetic processes of cotton leaves. – *Plant Physiol.* **83**: 360-364, 1987.
- Goltsev V., Zaharieva I., Chernev P. *et al.*: Drought-induced modifications of photosynthetic electron transport in intact leaves: Analysis and use of neural networks as a tool for a rapid non-invasive estimation. – *Biochim. Biophys. Acta* **1817**: 1490-1498, 2012.
- Govindjee, Amesz J., Fork D.C. (ed.): *Light Emission by Plants and Bacteria*. Pp. 660. Academic Press, Orlando 1986.
- Govindjee, Papageorgiou G.C.: Chlorophyll fluorescence and photosynthesis: fluorescence transients. – In: Giese A.C. (ed.): *Photophysiology*, Vol. 6. Pp. 1-46. Academic Press, New York 1971.
- Govindjee, Shevela D., Björn L.-O.: Evolution of the Z-scheme of photosynthesis: a perspective. – *Photosynth. Res.* **133**: 5-15, 2017.
- Govindjee: Chlorophyll *a* fluorescence: a bit of basics and history. – In: Papageorgiou G.C., Govindjee (ed.): *Chlorophyll *a* fluorescence: A signature of Photosynthesis, Advances in Photosynthesis and Respiration*. Vol. 19. Pp. 1-41. Springer, Dordrecht 2004.
- Govindjee: Sixty-three years since Kautsky: chlorophyll *a* fluorescence. – *Aust. J. Plant Physiol.* **22**: 131-160, 1995.
- Gravano E., Bussotti F., Strasser R.J. *et al.*: Ozone symptoms in leaves of woody plants in open-top chambers: ultrastructural and physiological characteristics. – *Physiol. Plantarum* **121**: 620-633, 2004.
- Greenbaum N.L., Ley A.C., Mauzerall D.C.: Use of a light-induced respiratory transient to measure the optical cross section of photosystem I in *Chlorella*. – *Plant Physiol.* **84**: 879-882, 1987.
- Guanter L., Zhang Y., Jung M. *et al.*: Global and time-resolved monitoring of crop photosynthesis with chlorophyll fluorescence. – *Proc. Natl. Acad. Sci. USA* **111**: E1327-E1333, 2014.
- Guisé B., Srivastava A., Strasser R.J.: The polyphasic rise of the chlorophyll *a* fluorescence (O-K-J-I-P) in heat stressed leaves. – *Arch. Sci. Genève* **48**: 147-160, 1995.
- Hakala M., Tuominen I., Keränen M.: Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of Photosystem II. – *Biochim. Biophys. Acta* **1706**: 68-80, 2005.
- Hamdani S., Qu M., Xin C.-P. *et al.*: Variations between the photosynthetic properties of elite and landrace Chinese rice cultivars revealed by simultaneous measurements of 820 nm transmission signal and chlorophyll *a* fluorescence induction. – *J. Plant Physiol.* **177**: 128-138, 2015.
- Hasegawa P.M., Bressan R.A., Zhu J. *et al.*: Plant cellular and molecular responses to high salinity. – *Annu. Rev. Plant Phys.* **51**: 463-499, 2000.
- Hendrickson L., Förster B., Pogson B.J. *et al.*: A simple chlorophyll fluorescence parameter that correlates with the rate coefficient of photoinactivation of photosystem II. – *Photosynth. Res.* **84**: 43-49, 2005.
- Hermans C., Smeyers M., Rodriguez R.M. *et al.*: Quality assessment of urban trees: A comparative study of physiological characterisation, airborne imaging and on site fluorescence monitoring by the OJIP-test. – *J. Plant Physiol.* **160**: 81-90, 2003.

- Hoagland D.R., Arnon D.I.: The water-culture method for growing plants without soil. – In: Agricultural Experiment Station, Circular 347. Pp. 1-39. College of Agriculture, University of California, Berkeley 1938.
- Humplik J.F., Lazár L., Husičková A. *et al.*: Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses – a review. – *Plant Methods* **11**: 29, 2015.
- Jedrowski C., Ashoub A., Momtaz O. *et al.*: Impact of drought, heat, and their combination on chlorophyll fluorescence and yield of wild barley (*Hordeum spontaneum*). – *J. Bot.* **2015**: 120868, 2015.
- Jedrowski C., Bayramov S., Brüggemann W.: Comparative analysis of drought stress effects on photosynthesis of Eurasian and North African genotypes of wild barley. – *Photosynthetica* **52**: 564-573, 2014.
- Jedrowski C., Brüggemann W.: Imaging of fast chlorophyll fluorescence induction curve (OJIP) parameters, applied in a screening study with wild barley (*Hordeum spontaneum*) genotypes under heat stress. – *J. Photoch. Photobio. B* **151**: 153-160, 2015.
- Jiang C.-D., Shi L., Gao H.-Y. *et al.*: Development of photosystems 2 and 1 during leaf growth in grapevine seedlings probed by leaf chlorophyll *a* fluorescence transient and 820 nm transmission *in vivo*. – *Photosynthetica* **44**: 454-463, 2006.
- Jiang H.-X., Chen L.-S., Zheng J.-G. *et al.*: Aluminum-induced effects on Photosystem II photochemistry in citrus leaves assessed by the chlorophyll *a* fluorescence transient. – *Tree Physiol.* **28**: 1863-1871, 2008.
- Jiang H.-X., Tang N., Zheng J.-G. *et al.*: Antagonistic actions of boron against inhibitory effects of aluminum toxicity on growth, CO<sub>2</sub> assimilation, ribulose-1,5-bisphosphate carboxylase/oxygenase, and photosynthetic electron transport probed by the JIP-test, of *Citrus grandis* seedlings. – *BMC Plant Biol.* **9**: 102, 2009.
- Joshi M.K., Mohanty P.: Chlorophyll *a* fluorescence as a probe of heavy metal ion toxicity in plants. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Vol. 19. Pp. 637-661. Springer, Dordrecht 2004.
- Kalaji H.M., Carpentier R., Allakhverdiev S.I. *et al.*: Fluorescence parameters as early indicators of light stress in barley. – *J. Photoch. Photobio. B* **112**: 1-6, 2012.
- Kalaji H.M., Jajoo A., Oukarroum A. *et al.*: Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. – *Acta Physiol. Plant.* **38**: 102, 2016.
- Kalaji H.M., Oukarroum A., Alexandrov V. *et al.*: Identification of nutrient deficiency in maize and tomato plants by *in vivo* chlorophyll *a* fluorescence measurements. – *Plant Physiol. Biochem.* **81**: 16-25, 2014b.
- Kalaji H.M., Schansker G., Brestic M. *et al.*: Frequently asked questions about chlorophyll fluorescence, the sequel. – *Photosynth. Res.* **132**: 13-66, 2017a.
- Kalaji H.M., Schansker G., Ladle R.J. *et al.*: Frequently asked questions about chlorophyll fluorescence: practical issues. – *Photosynth. Res.* **122**: 121-158, 2014a.
- Kalaji M.H., Goltsev V.N., Żuk-Gołaszewska K. *et al.*: Chlorophyll Fluorescence: Understanding Crop Performance – Basics and Applications. Pp. 222. CRC Press, Boca Raton 2017b.
- Kale R., Hebert A.E., Frankel L.K. *et al.*: Amino acid oxidation of the D1 and D2 proteins by oxygen radicals during photoinhibition of Photosystem II. – *Proc. Natl. Acad. Sci. USA* **114**: 2988-2993, 2017.
- Kaňa R., Govindjee: Role of ions in the regulation of light harvesting. – *Front. Plant Sci.* **7**: 1849, 2016.
- Kouřil R., Lazár D., Lee H. *et al.*: Moderately elevated temperature eliminates resistance of rice plants with enhanced expression of glutathione reductase to intensive photooxidative stress. – *Photosynthetica* **41**: 571-578, 2003.
- Krause G.H., Weis E.: Chlorophyll fluorescence and photosynthesis: the basics. – *Annu. Rev. Plant Phys.* **42**: 313-349, 1991.
- Kromdijk J., Głowacka K., Leonelli L. *et al.*: Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. – *Science* **354**: 857-861, 2016.
- Lazár D., Ilík P., Nauš J.: An appearance of K-peak in fluorescence induction depends on the acclimation of barley leaves to higher temperatures. – *J. Lumin.* **72-74**: 595-596, 1997.
- Lazár D., Ilík P.: High-temperature induced chlorophyll fluorescence changes in barley leaves. Comparison of the critical temperatures determined from fluorescence induction and from fluorescence temperature curve. – *Plant Sci.* **124**: 159-164, 1997.
- Lazár D., Nauš J.: Statistical properties of chlorophyll fluorescence induction parameters. – *Photosynthetica* **35**: 121-127, 1998.
- Lazár D., Pospíšil P., Nauš J.: Decrease of fluorescence intensity after the K step in chlorophyll *a* fluorescence induction is suppressed by electron acceptors and donors to photosystem 2. – *Photosynthetica* **37**: 255-265, 1999.
- Lazár D., Schansker G.: Models of chlorophyll *a* fluorescence transients. – In: Laisk A., Nedbal A.L., Govindjee (ed.): Photosynthesis *in Silico*: Understanding Complexity from Molecules to Ecosystems. Advances in Photosynthesis and Respiration. Vol. 29. Pp. 85-123. Springer, Dordrecht 2009.
- Lazár D.: Chlorophyll *a* fluorescence induction. – *Biochim. Biophys. Acta* **1412**: 1-28, 1999.
- Lazár D.: Chlorophyll *a* fluorescence rise induced by high light illumination of dark-adapted plant tissue studied by means of a model of photosystem II and considering photosystem II heterogeneity. – *J. Theor. Biol.* **220**: 469-503, 2003.
- Lazár D.: Modelling of light-induced chlorophyll *a* fluorescence rise (O–J–I–P transient) and changes in 820 nm-transmittance signal of photosynthesis. – *Photosynthetica* **47**: 483-498, 2009.
- Lazár D.: Parameters of photosynthetic energy partitioning. – *J. Plant Physiol.* **175**: 131-147, 2015.
- Lazár D.: Simulations show that a small part of variable chlorophyll *a* fluorescence originates in photosystem I and contributes to overall fluorescence rise. – *J. Theor. Biol.* **335**: 249-264, 2013.
- Lazár D.: The polyphasic chlorophyll *a* fluorescence rise measured under high intensity of exciting light. – *Funct. Plant Biol.* **33**: 9-30, 2006.
- Ley A.C., Mauzerall D.C.: Absolute absorption cross-sections for photosystem II and the minimum quantum requirement of photosynthesis in *Chlorella vulgaris*. – *Biochim. Biophys. Acta* **680**: 95-106, 1982.
- Liang Y., Chen H., Tang M.J. *et al.*: Responses of *Jatropha curcas* seedlings to cold stress: photosynthesis-related proteins and chlorophyll fluorescence characteristics. – *Physiol. Plantarum* **131**: 508-517, 2007.
- Lichtenthaler H.K., Buschmann C., Rinderle U. *et al.*: Application of chlorophyll fluorescence in ecophysiology. – *Radiat. Environ. Biophys.* **25**: 297-308, 1986.
- Liu Q.D., Zhu Y.R., Tao H.L. *et al.*: Damage of PSII during senescence of *Spirodela polyrrhiza* explants under long-day



- conditions and its prevention by 6-benzyladenine. – *J. Plant Res.* **119**: 145-152, 2006.
- Makino A.: Rubisco and nitrogen relationships in rice: Leaf photosynthesis and plant growth. – *Soil Sci. Plant Nutr.* **49**: 317-327, 2003.
- Marschner H.: *Mineral Nutrition of Higher Plants*, 2<sup>nd</sup> ed. Academic Press, London 1995.
- Mathur S., Jajoo A., Mehta P. *et al.*: Analysis of elevated temperature-induced inhibition of photosystem II using chlorophyll a fluorescence induction kinetics in wheat leaves (*Triticum aestivum*). – *Plant Biol.* **13**: 1-6, 2011.
- McGrath J.M., Bezelberger A.M., Wang S. *et al.*: An analysis of ozone damage to historical maize and soybean yields in the United States. – *Proc. Natl. Acad. Sci. USA* **112**: 14390-14395, 2015.
- Mehta P., Jajoo A., Mathur S. *et al.*: Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. – *Plant Physiol. Biochem.* **48**: 16-20, 2010.
- Meroni M., Rossini M., Guanter L. *et al.*: Remote sensing of solar-induced chlorophyll fluorescence: Review of methods and applications. – *Remote Sens. Environ.* **113**: 2037-2051, 2009.
- Mishra K.B., Mishra A., Klem K. *et al.*: Plant phenotyping: a perspective. – *Ind. J. Plant Physiol.* **21**: 514-527, 2016a.
- Mishra K.B., Mishra A., Novotná K. *et al.*: Chlorophyll a fluorescence, under half of the adaptive growth-irradiance, for high-throughput sensing of leaf-water deficit in *Arabidopsis thaliana* accessions. – *Plant Methods* **12**: 46, 2016b.
- Misra A.N. Srivastava A., Strasser R.J.: Utilization of fast chlorophyll a fluorescence technique in assessing the salt/ion sensitivity of mung bean and *Brassica* seedlings. – *J. Plant Physiol.* **158**: 1173-1181, 2001.
- Morales F., Abadía A., Abadía J.: Photoinhibition and photoprotection under nutrient deficiencies, drought and salinity. – In: Demmig-Adams B., Adams III W.W., Mattoo A.K. (ed.): *Photoprotection, Photoinhibition, Gene Regulation, and Environment*. Pp. 65-85. Springer Science+Business Media B.V. Dordrecht 2008.
- Moya I., Cerovic Z.G.: Remote sensing of chlorophyll fluorescence: instrumentation and analysis. – In: Papageorgiou G.C., Govindjee (ed.): *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Pp. 429-445. Springer, Dordrecht 2004.
- Müller P., Li X.-P., Niyogi K.K.: Non-photochemical quenching. A response to excess light energy. – *Plant Physiol.* **125**: 1558-1566, 2001.
- Munday J.C. Jr., Govindjee: Light-induced changes in the fluorescence yield of chlorophyll a *in vivo*. III. The dip and the peak in the fluorescence transient of *Chlorella pyrenoidosa*. – *Biophys. J.* **9**: 1-21, 1969a.
- Munday J.C. Jr., Govindjee: Light-induced changes in the fluorescence yield of chlorophyll a *in vivo*. IV. The effect of preillumination on the fluorescence transient of *Chlorella pyrenoidosa*. – *Biophys. J.* **9**: 22-35, 1969b.
- Munns R., Tester M.: Mechanisms of salinity tolerance. – *Annu. Rev. Plant Biol.* **59**: 651-681, 2008.
- Murata N., Takahashi S., Nishiyama Y. *et al.*: Photoinhibition of photosystem II under environmental stress. – *Biochim. Biophys. Acta* **1767**: 414-421, 2007.
- Murchie E.H., Lawson T.: Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – *J. Exp. Bot.* **64**: 3983-3998, 2013.
- Nagajyoti P.C., Lee K.D., Sreekanth T.V.M.: Heavy metals, occurrence and toxicity for plants: a review. – *Environ. Chem. Lett.* **8**: 199-216, 2010.
- Nash D., Miyao M., Murata N.: Heat inactivation of oxygen evolution in photosystem II particles and its acceleration by chloride depletion and exogenous manganese. – *Biochim. Biophys. Acta* **807**: 127-133, 1985.
- Nauš J., Kuropatwa R., Klinkovský T. *et al.*: Heat injury of barley leaves detected by the chlorophyll fluorescence temperature curve. – *Biochim. Biophys. Acta* **1101**: 359-362, 1992.
- Nernst W.H.: [Kinetics of solids: theory of diffusion.] – *Z. Phys. Chem.* **3**: 613-637, 1888. [In German]
- Niinemets U.: A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. – *Ecol. Res.* **25**: 693-714, 2010.
- Nikiforou C., Manetas Y.: Inherent nitrogen deficiency in *Pistacia lentiscus* preferentially affects photosystem I: a seasonal field study. – *Funct. Plant Biol.* **38**: 848-855, 2011.
- Nilkens M., Kress E., Lambrev P. *et al.*: Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in *Arabidopsis*. – *Biochim. Biophys. Acta* **1797**: 466-475, 2010.
- Oukarroum A., El Madidi S., Schansker G. *et al.*: Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering. – *Environ. Exp. Bot.* **60**: 438-446, 2007.
- Oukarroum A., El Madidi S., Strasser R.J.: Differential heat sensitivity index in barley cultivars (*Hordeum vulgare* L.) monitored by chlorophyll a fluorescence OKJIP. – *Plant Physiol. Biochem.* **105**: 102-108, 2016.
- Oukarroum A., Strasser R.J., van Staden J.: Phenotyping of dark and light adapted barley plants by the fast chlorophyll a fluorescence rise OJIP. – *S. Afr. J. Bot.* **70**: 277-283, 2004.
- Pan X., Chen X., Zhang D. *et al.*: Effect of Chromium(VI) on photosystem II activity and heterogeneity of *Synechocystis* sp. (Cyanophyta): studied with *in vivo* chlorophyll fluorescence tests. – *J. Phycol.* **45**: 386-394, 2009.
- Paoletti E., Bussotti F., Della Rocca G. *et al.*: Fluorescence transient in ozonated Mediterranean shrubs. – *Phyton* **44**: 121-131, 2004.
- Papageorgiou G.C., Govindjee (ed.): *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. *Advances in Photosynthesis and Respiration*, Vol. 19. Pp. 820. Springer, Dordrecht 2004.
- Papageorgiou G.C., Govindjee: Photosystem II fluorescence: slow changes - scaling from the past. – *J. Photochem. Photobiol. B.* **104**: 258-270, 2011
- Papageorgiou G.C., Tsimilli-Michael M., Stamatakis K.: The fast and slow kinetics of chlorophyll a fluorescence induction in plants, algae and cyanobacteria: a viewpoint. – *Photosynth. Res.* **94**: 275-290, 2007.
- Pareek A., Sopory S.K., Bohnert H.K. *et al.* (ed.): *Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation*. Pp. 526, Springer, Dordrecht 2010.
- Parida A.K., Das A.B.: Salt tolerance and salinity effects on plants: A review. – *Ecotoxicol. Environ. Safe.* **60**: 324-349, 2005.
- Prakash J.S.S., Srivastava A., Strasser R.J. *et al.*: Senescence induced alterations in the photosystem II functions of *Cucumis sativus* cotyledons: probing of senescence driven alterations of photosystem II by chlorophyll a fluorescence induction O-J-I-P transients. – *Indian J. Biochem. Biophys.* **40**: 160-168, 2003.
- Rapacz M., Sasal M., Kalaji H.M. *et al.*: Is the OJIP test a reliable indicator of winter hardiness and freezing tolerance of common

- wheat and triticale under variable winter environments? – *PLoS ONE* **10**: e0134820, 2015b.
- Rapacz M., Sasal M., Wójcik-Jagła M.: Direct and indirect measurements of freezing tolerance: advantages and limitations. – *Acta Physiol. Plant.* **37**: 157-173, 2015a.
- Rapacz M., Woźniczka A.: A selection tool for freezing tolerance in common wheat using the fast chlorophyll *a* fluorescence transient. – *Plant Breeding* **128**: 227-234, 2009.
- Rapacz M.: Chlorophyll *a* fluorescence transient during freezing and recovery in winter wheat. – *Photosynthetica* **45**: 409-418, 2007.
- Schansker G., Tóth S.Z., Holzwarth A.R. *et al.*: Chlorophyll *a* fluorescence: beyond the limits of the Q<sub>A</sub>-model. – *Photosynth. Res.* **120**: 43-58, 2014.
- Schansker G., Tóth S.Z., Kovács L. *et al.*: Evidence for a fluorescence yield change driven by a light induced conformational change within photosystem II during the fast chlorophyll *a* fluorescence rise. – *Biochim. Biophys. Acta* **1807**: 1032-1043, 2011.
- Schreiber U., Berry J.A.: Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of photosynthetic apparatus. – *Planta* **136**: 233-238, 1977.
- Serbin S.P., Dillaway D.N., Kruger E.L. *et al.*: Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. – *J. Exp. Bot.* **63**: 489-502, 2012.
- Shabnam N., Sharmila P., Govindjee *et al.*: Differential response of floating and submerged leaves of long leaf pondweed to silver ions. – *Front. Plant Sci.* **8**: 1052, 2017.
- Srivastava A., Govindjee, Strasser R.J.: Greening of peas: parallel measurements on 77 K emission spectra, OJIP chlorophyll *a* fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. – *Photosynthetica* **37**: 365-392, 1999.
- Srivastava A., Guissé B., Greppin H. *et al.*: Regulation of antenna structure and electron transport in PSII of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll *a* fluorescence transient OKJIP. – *Biochim. Biophys. Acta* **1320**: 95-106, 1997.
- Stauffer P.H.: Flux flumoxed: A proposal for consistent usage. – *Ground Water* **44**: 125-128, 2006.
- Stefanov D., Petkova V., Denev I.D.: Screening for heat tolerance in common bean (*Phaseolus vulgaris* L.) lines and cultivars using JIP-test. – *Sci. Hortic.-Amsterdam* **128**: 1-6, 2011.
- Stirbet A., Govindjee, Strasser B.J. *et al.*: Chlorophyll *a* fluorescence induction in higher plants: Modelling and numerical simulation. – *J. Theor. Biol.* **193**: 131-151, 1998.
- Stirbet A., Govindjee: Chlorophyll *a* fluorescence induction: a personal perspective of the thermal phase, the J-I-P rise. – *Photosynth. Res.* **113**: 15-61, 2012.
- Stirbet A., Govindjee: On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and photosystem II: Basis and applications of the OJIP fluorescence transient. – *J. Photochem. Photobiol. B* **104**: 236-257, 2011.
- Stirbet A., Riznichenko G.Yu., Rubin A.B. *et al.*: Modeling chlorophyll *a* fluorescence transient: relation to photosynthesis. – *Biochemistry-Moscow* **79**: 291-323, 2014.
- Strasser B.J., Strasser R.J.: Measuring fast fluorescence transients to address environmental questions: The JIP test. – In: Mathis P. (ed.): *Photosynthesis: from Light to Biosphere*. Vol. 5. Pp. 977-980. Kluwer Academic Publishers, Dordrecht 1995.
- Strasser B.J.: Donor side capacity of photosystem II probed by chlorophyll *a* fluorescence transients. – *Photosynth. Res.* **52**: 147-155, 1997.
- Strasser R.J., Srivastava A., Govindjee: Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. – *Photochem. Photobiol.* **61**: 32-42, 1995.
- Strasser R.J., Srivastava A., Tsimilli-Michael M.: Screening the vitality and photosynthetic activity of plants by fluorescence transient. – In: Behl R.K., Punia M.S., Lather B.P.S. (ed.): *Crop Improvement for Food Security*. Pp. 72-115. SSARM, Hisar, India 1999.
- Strasser R.J., Tsimilli-Michael M., Dangre D. *et al.*: Biophysical phenomics reveals functional building blocks of plants systems biology: a case study for the evaluation of the impact of mycorrhization with *Piriformospora indica*. – In: Varma A., Oelmüller R. (ed.): *Advanced Techniques in Soil Microbiology*. Soil Biology. Pp. 319-341. Springer, Berlin 2007.
- Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (ed.): *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis, Advances in Photosynthesis and Respiration*, Vol. 19. Pp. 321-362. Springer, Dordrecht 2004.
- Strasser R.J., Tsimilli-Michael M., Srivastava A.: The fluorescence transient as a tool to characterize and screen photosynthetic samples. – In: Yunus M., Pathre U., Mohanty P. (ed.): *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*. Pp. 443-480. Taylor & Francis, London 2000.
- Strasser R.J.: The grouping model of plant photosynthesis. – In: Akoyunoglou G., Argyroudi-Akoyunoglou J.H. (ed.): *Chloroplast Development*. Pp. 513-538. Elsevier Biomedical, Amsterdam 1978.
- Strauss A.J., Krüger G.H.J., Strasser R.J. *et al.*: Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll *a* fluorescence transient O-J-I-P. – *Environ. Exp. Bot.* **56**: 147-157, 2006.
- Sudhir P., Murthy S.D.S.: Effects of salt stress on basic processes of photosynthesis. – *Photosynthetica* **42**: 481-486, 2004.
- Toepel J., Gilbert M., Wilhelm C.: Can chlorophyll *a* *in-vivo* fluorescence be used for quantification of carbon-based primary production in absolute terms? – *Arch. Hydrobiol.* **160**: 515-526, 2004.
- Tsimilli-Michael M., Eggenberg P., Biro B. *et al.*: Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and *Azospirillum* and *Rhizobium* nitrogen-fixers on the photosynthetic activity of alfalfa, probed by the polyphasic chlorophyll *a* fluorescence transient O-J-I-P. – *Appl. Soil Ecol.* **15**: 169-182, 2000.
- Tsimilli-Michael M., Pêcheux M., Strasser R.J.: Vitality and stress adaptation of the symbionts of coral reef and temperate foraminifers probed *in hospite* by the fluorescence kinetics O-J-I-P. – *Arch. Sci. Genève* **51**: 1-36, 1998.
- Tsimilli-Michael M., Strasser R.J.: *In vivo* assessment of plants' vitality: applications in detecting and evaluating the impact of mycorrhization on host plants. – In: Varma A. (ed.): *Mycorrhiza: State of the Art. Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics*, 3<sup>rd</sup> ed. Pp. 679-703. Springer, Dordrecht 2008.
- van Heerden P.D.R., Strasser R.J., Krüger G.H.J.: Reduction of dark chilling stress in N<sub>2</sub>-fixing soybean by nitrate as indicated by chlorophyll *a* fluorescence kinetics. – *Physiol. Plantarum* **121**: 239-249, 2004b.
- van Heerden P.D.R., Tsimilli-Michael M., Krüger G.H.J. *et al.*: Dark chilling effects on soybean genotypes during vegetative development: Parallel studies of CO<sub>2</sub> assimilation, chlorophyll *a* fluorescence kinetics O-J-I-P and nitrogen fixation. – *Physiol. Plantarum* **117**: 476-491, 2003.

- van Heerden P.D.R., Viljoen M.M., DeVilliers M. *et al.*: Limitation of photosynthetic carbon metabolism by dark chilling in temperate and tropical soybean genotypes. – *Plant Physiol. Biochem.* **42**: 117-124, 2004a.
- van Straten G., van Thoor B., van Willegenburg L.G. *et al.*: A ‘big leaf, big fruit, big substrate’ model for experiments on receding horizon optimal control of nutrient supply to greenhouse tomato. – *Acta Hort.* **718**: 147-155, 2006.
- Volgusheva A., Yakovleva O.V., Kukarskikh G.P. *et al.*: Performance index in assessing the physiological state of trees in urban ecosystems. – *Biophysics* **56**: 90-95, 2011.
- Wang X.Y., Xu X.M., Cui J.: The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light. – *Photosynthetica* **53**: 213-222, 2015.
- Wong D., Govindjee: Effects of lead ions on photosystem I in isolated chloroplasts: Studies on the reaction center P700. – *Photosynthetica* **10**: 241-254, 1976.
- Yan K., Chen P., Shao H. *et al.*: Responses of photosynthesis and photosystem II to higher temperature and salt stress in sorghum. – *J. Agron. Crop Sci.* **198**: 218-226, 2012.
- Yusuf M.A., Kumar D., Rajwanshi R. *et al.*: Overexpression of gamma-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: Physiological and chlorophyll fluorescence measurements. – *Biochim. Biophys. Acta* **1797**: 1428-1438, 2010.
- Yusuf M.A., Sarin N.B.: Antioxidant value addition in human diets: genetic transformation of *Brassica juncea* with  $\gamma$ -TMT gene for increased  $\alpha$ -tocopherol content. – *Transgenic Res.* **16**: 109-113, 2007.
- Zhu X.-G., Govindjee, Baker N.R. *et al.*: Chlorophyll *a* fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with Photosystem II. – *Planta* **223**: 114-133, 2005.
- Zubek S., Stojakowska A., Anielska T. *et al.*: Arbuscular mycorrhizal fungi alter thymol derivative contents of *Inula ensifolia* L. – *Mycorrhiza* **20**: 497-504, 2010.
- Živčák M., Olšovská K., Slamka P. *et al.*: Measurements of chlorophyll fluorescence in different leaf positions may detect nitrogen deficiency in wheat. – *Zemdirbyste-Agriculture* **101**: 437-443, 2014.