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Research Article

USE OF O-J-I-P CHLOROPHYLL FLUORESCENCE TRANSIENTS TO PROBE MULTIPLE EFFECTS OF UV-C RADIATION ON THE PHOTOSYNTHETIC APPARATUS OF *EUGLENA*

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Abstract

Although the kinetic chlorophyll fluorescence signals are rich in information, most of the chlorophyll fluorescence related studies deal only with the quantum yield of primary photochemistry (F_v/F_m). JIP-test based OJIP fluorescence transient analysis is relatively a new technique to investigate the environmental stress responses of photosynthetic organisms. In the present study, the deleterious effects of ultraviolet (UV) radiation on the photosynthetic machinery were probed by the JIP-test in *Euglena*, one of the most potent organisms for the future space stations. The cells were exposed to a series of UV-C doses and immediately after exposure, survival percentage was determined with Neutral Red staining, and the chlorophyll fluorescence was measured using AquaPen AP-C 100 fluorometer. Resultant OJIP transients were analyzed according to JIP-test, and several functional and structural parameters were derived to explain the PSII behavior. Results indicated that the UV-C induced inhibition of electron transport is severely affected due to higher sensitivity of dark reactions after Q_A^-, represented as ψ_o, the electron transfer probability, than of the light dependent reactions, represented as φ_{Po}, the trapping probability. The performance index (PI_{ABS}) of PSII, which is a combination of the indices of three independent parameters, decreased markedly in exponential manner in response to UV-C. Results illustrate the advantage of using a number of fluorescent parameters over the use of one parameter, often the F_v/F_m.

Keywords: JIP-test; performance index; PS II; quantum yield

Introduction

Chlorophyll fluorescence emitted by sensitive photosynthetic organisms reflects the energetic behavior of photosynthetic system. It has become an important tool in monitoring the photosynthetic events and analyzing the physiological state of photosynthetic organisms. Moreover, chlorophyll fluorescence transient and its measurements are strong enough to provide reliable interpretation (Force *et al*., 2003) on the complex signals by facilitating deeper insight into the mechanism of fluorescence emission.

In photosynthesis, antenna pigments absorb the light and excitation energy is transferred to the reaction centers (RC) of the two photo-systems (PS I and II) (Krause and Weis, 1991). Absorbed light energy can undergo one of photochemistry, heat, or fluorescence (through photo-emission) (Misra *et al*., 2012). Although these processes compete each other total energy is constant. In increase in one process will decrease the yield of the other two processes. Thus, measurement in yield of chlorophyll fluorescence gives information about the efficiency of photochemistry and heat dissipation. Among PS I and II, PS II has been identified as the most liable component of the photosynthetic apparatus (Lazar, 1999 and Govidgee, 2004).

The energy flux theory in photosynthetic apparatus is the function of a system mainly based on the Butler bipartite, where it treats the antennae and reaction centre separately, of variable PS II fluorescence (Strasser, 1981, 1978). Accordingly , it has been proposed four types of energy fluxes as an absorbed flux (ABS), a trapping flux (TR), an electron flux (ET) and a dissipation flux (DI), the non trapped energy emit as heat and fluorescence (Fig. 1).

Strasser and Strasser (1995) introduced the JIP test which is a simple energy flux theory, that translates the changes observed in the fluorescent transients to quantitative changes in formulated parameters (Force *et al.*, 2003). This OJIP curve has been derived from the exponential phase of the typical Kautsky curve or OPS curve (Kautsky and Hirsh, 1931) after inserted to a logarithmic time scale. Then, it has revealed a shape with many phases and rich in information about the behavior of the photosynthetic apparatus being at any physiological state (Strasser *et al.*, 2004).

Nevertheless, the JIP test is a stepwise flow of energy (Fig. 2) through PS II at the reaction centre (RC) level as well as the level of a cross section (CS). The maximum quantum

yield of primary photochemistry ($F_v/F_m = \varphi_{Po}$) is just one of parameters that can be derived from it. Although kinetic Chlorophyll fluorescence signal carries enormous amount of information, many scientists still rely on the F_v/F_m ratio in the photosynthetic pathway. The analysis of the O-J-I-P polyphasic transients according to the JIP-test is becoming popular as this rapid and non-invasive analysis allows deriving several expressions leading to a detailed description of a photosynthetic organism at a given physiological state (Strasser *et al.*, 2000).

Euglena is a unicellular photosynthetic organism which has been proposed as a valuable biological component to be used in closed ecological life-support systems such as space stations due to its high photosynthetic efficiency and nutritional quality. The substantially high resistance of this organism to UV radiation adds more value in this respect.

Even though photosynthetic pigments are bleached and destroyed by higher doses of UV radiation, the vulnerable targets in the photosynthetic pathway has not been clearly identified in most organisms. Hence, the present study was conducted to probe the deleterious effects of ultraviolet radiation on the photosynthetic machinery of *Euglena* using OJIP chlorophyll fluorescent transients followed by the JIP-test, a relatively new technique.

Materials and Methods

Biological culture and UV-C exposure

Euglena gracilis Klebs strain Z was cultured photoautotrophically in modified Cramer-Mayer medium according to Bolige *et al.* (2006) at room temperature or 25°C. Exponentially growing cells were exposed to UV radiation; a series of doses was administered using a UV-C germicidal lamp (peak = 254 nm; GL-15, Panasonic, Tokyo, Japan) with an intensity of 2.2 Wm^{-2}, while changing the exposure time.

Chlorophyll fluorescence measurements

Immediately after exposing to the UV-C, survival percentage was determined with Neutral Red staining, and the chlorophyll fluorescence was measured in cell suspensions (2 ml) using AquaPen AP-C 100 fluorometer (PSI, Czech Republic). For each measurement, a cell titer of ~ 8 × 104 cells/ml was used. The samples were dark-adapted for 10 min. dark-adaptation, the cells were exposed to a saturating light pulse of 3000 µmolm-2s-1. The fluorescent transients were recorded in a time span from 10 µs to 1 s at 10 µs intervals.

Analysis of chlorophyll fluorescence

Each transient was analyzed according to JIP-test (Strasser *et al.*, 2000) by using raw data; F_0 - the fluorescence intensity at 50 µs when all RCs are open (O-step), F_K - at 300 µs (K-step), F_J - at 2 ms (J-step), F_I - at 30 ms (I-step) and F_M - maximum fluorescence intensity assuming all the RCs are closed by the saturating light pulse (P-step). Using these values in equations purposed by the JIP-test, it was converted the experimental fluorescence signals into bio-energetic meaning and several parameters were calculated (Fig. 2). Two other basic parameters were also derived (Equations and definitions of JIP parameters by Strasser *et al* (2004; 2010) ;

Net rate of PS II closure: an approximation of the slope at the origin of the fluorescence rise $(dF/dt)_0$, is the measure of the rate of the primary photochemistry (Eqn.1). It is a net rate because the reduced PS II primary electron accepter (Q_A) can be re-oxidized via electron transport beyond Q_A.

$$M_0 = 4(F_K-F_0) / (F_M-F_0) \quad \text{Eqn.1}$$

Relative variable fluorescence at 2 ms

For connected PS II units, equals the fraction of closed RCs at 2 ms expressed as a proportion of the total number of RCs that can be closed (Eqn.2).

$$V_J = (F_J-F_0) / (F_M-F_0) \quad \text{Eqn.2}$$

Following equations were used to explain the PSII behavior:

(a) Specific energy fluxes (per RC) for absorption (ABS/RC), trapping (TR_0/RC), electron transport (ET_0/RC) and dissipation (DI_0/RC). These fluxes are interrelated and outlined as a pipeline model in Fig. 1.

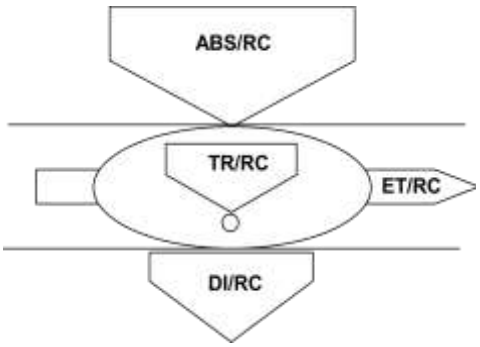

Fig. 1: Energy pipeline membrane model

Simplified scheme for the interrelation of specific energy fluxes (per RC) for absorption (ABS/RC), trapping (TR_0/RC), electron transport (ET_0/RC) and dissipation (DI_0/RC) in the photosynthetic apparatus

a. *Effective antenna size of an active RC:* the total number of photons absorbed by chlorophyll molecules of all RCs divided by the total number of the active RCs. It influenced by the ratio of active/inactive RCs.

$$ABS/RC = (M_0/V_j) / [1–(F_0/F_m)] \quad \text{Eqn.3}$$

b. *Electron transport in an active RC:* the re-oxidation of reduced Q_A via electron transport in an active RC. Only reflect the activity of active RCs.

$$ET_0/RC = (M_0/V_j) \times (1–V_j) \quad \text{Eqn.4}$$

c. *Maximal trapping rate of PS II:* the maximal rate by which an exciton is trapped by the RC resulting in the reduction of Q_A.

$$TR_0/RC = (M_0/V_j) \quad \text{Eqn.5}$$

d. *Effective dissipation of an active RC:* the ratio of the total dissipation of un-trapped excitation energy from all RCs with respect to the number of active RCs. It influenced by the ratio of active/inactive RCs.

$$DI_0/RC = (ABS/RC) – (TR_0/RC) \quad \text{Eqn.6}$$

(b) Flux ratios or yields, i.e. maximum quantum yield of primary photochemistry (φ_{Po}), electron transport probability (ψ_o), and the quantum yield of electron transport (φ_{Eo}):

$$\varphi_{Po} = [1–(F_0/F_m)] = TR_0/AB = F_V/F_m \quad \text{Eqn.7}$$

$$\psi_o = (1–V_j) = ET_0/TR_0 \quad \text{Eqn.8}$$

$$\varphi_{Eo} = [1–(F_0/F_m)] \times (1–V_j) = ET_0/ABS \quad \text{Eqn.9}$$

(c) Performance Index (PI):

$$PI_{ABS} = (RC/ABS) \times (\varphi_{Po}/(1-\varphi_{Po})) \times (\psi_o/(1-\psi_o)) \quad \text{Eqn. 10}$$

Results and Discussion

LD_{50} of UV-C

UV radiation which is a part of the solar spectrum contains UV-C, UV-B and UV-A. The effect of UV radiation on photosynthetic organisms has been extensively investigated over the past decades due to the depletion of ozone layer. Many studies have analyzed the effect on UV radiation on photosynthetic organisms and observed that UV radiation causes serious damages to photosynthetic apparatus thus, induced a decrease of the photosynthetic capacity (Prasad and Zeeshan (2004), Holzinger and Lutz (2005) and Takahashi *et al.* (2006)).

In this study, the LD_{50} of UV-C radiation for *E. gracilis* was found to be ~ 1.2 KJ/m^2. Until *ca*. 0.5 KJ/m^2, a significant cell death was not observed (Figure 3).

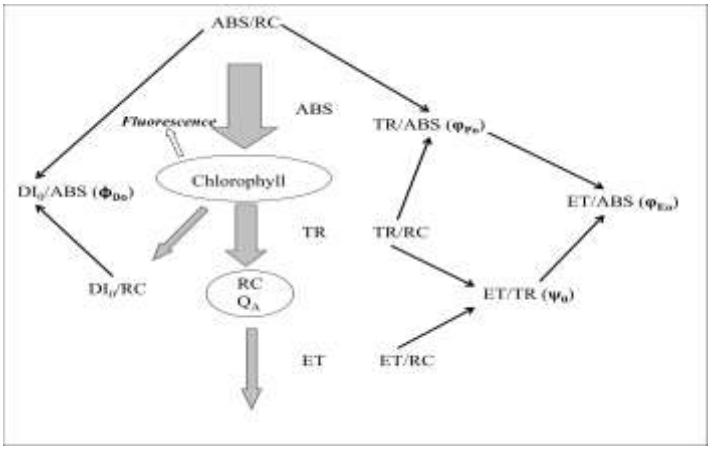

Fig. 2: The outline of the JIP parameters

The stepwise flow of energy through PS II at both reaction centre (RC) level (absorption (ABS/RC), trapping (TR_0/RC), electron transport (ET_0/RC) and dissipation (DI_0/RC)) and the level of cross section (CS) ((ABS/RC), (TR_0/RC), (ET_0/RC) and (DI_0/RC)). The parameters are interrelated by probabilities that define exciton trapping (TR_0/ABS) and electron transport (ET_0/ TR_0).

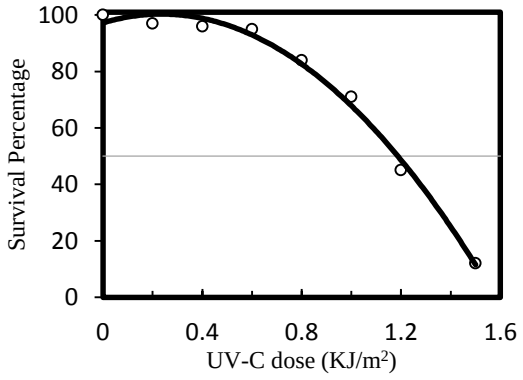

Fig. 3: The LD_{50} of UV-C radiation for *E. gracilis*

A significant cell death shows after 0.5 KJ/m^2 and LD_{50} of UV-C for *E. gracilis* observed at 1.2 KJ/m^2

In this experiment, *E. gracilis* was exposed to a series of UV-C doses and, the results showed a significant increment of the measured JIP fluorescence intensities at 0.05 ms (F_0), at 0.3 ms (F_K), at 2 ms (F_J), at 30 ms (F_I), and at the saturating light pulse (F_M) (data not shown). Clark *et al.* (2000) and Kuger *et al.* (1997) reported that the performance of the PS II and the impact of stress conditions can be detected using the JIP-test as it revealed information based on different scales.

Specific energy fluxes

Specific energy fluxes (Fig. 4) show that an increase in UV-C dose results in an increase in the effective antenna size of an active reaction center and a decrease in electron transport flux, while keeping the maximum rate by which an exciton is trapped by the RC, unchanged. These changes have led to an increase in the dissipation flux. The change in the antenna size of PSII can be due to the inactivation of RCs by UV radiation. On the other way around, as the antenna size is calculated average values as total absorbing chlorophyll per total fully active PS II RCs, and it can be increased if RCs were converted into heat sinks or other regrouping of antennae from active RCs to inactive RCs occurred (Strasser *et al.*, 1995 and Van Heerden *et al.*, 2003). Force *et al.* (2003) stated that dissipation can be thought of as the absorption of photons in excess of what can be trapped by the RC. Dissipation also results the decrease level of electron transport.

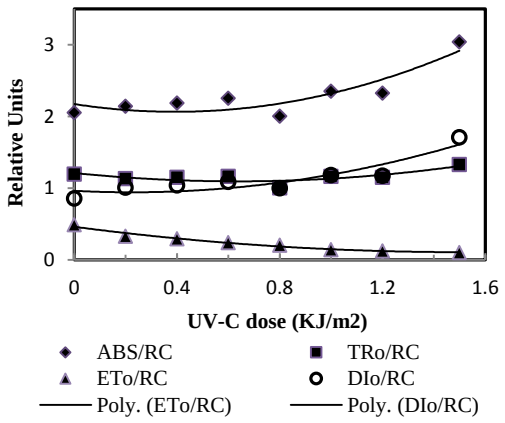

Fig. 4: Specific energy fluxes (per RC)
An increase in UV-C dose results increased in absorption (ABS/RC), unchanged trapping (TR_0/RC), decreased electron transport (ET_0/RC) and increased dissipation (DI_0/RC).

Moreover, UV radiation also lowered the photosynthetic performance of a single RC as can be seen by the decreased electron transport flux (Fig. 4).

Quantum efficiencies

The quantum efficiencies; ET_0/ABS, ET_0/TR_0 and TR_0/ABS_0, analyzed in the present study, decreased with the increasing UV-C dose (Fig. 5). However, ψ_o (Eqn.8) that expresses the efficiency of a trapped exciton that can move an electron into the electron transport chain further than Q_A^- seemed severely affected compared to other two flux ratios, i.e. φ_{Po} (Eqn.7) and φ_{Eo} (Eqn.9). Furthermore, a reduction in the electron transport capacity (ET_0/TR_0) was resulted due to the decline in ET_0/RC (Eqn.4). This behavior indicates that the UV-C induced inhibition of electron transport is more due to ψ_o; highly dark sensitive reactions after Q_A^-, than φ_{Po}; the light dependent reactions.

The increase in DI_0/RC and the decline in ET_0/TR_0 may indicate that the UV exposed *E. gracilis* was photoinhibited. Similar results were reported by Wobese *et al.* (2000), an increase in photoinhibition as a consequence of high exposure to UV-B radiation in *Macrocystis pyrifera* and a more pronounced photoinhibition under UV-B in *Porphyra umumbilicalis* (Aguilera *et al.*, 1999). Lowering of the F_V/F_M ratio (TR_0/ABS) has been the most prominent parameter to measure the photoinhibition to date. However, it may also be accompanied by an increase in the minimum fluorescence level, but not always as reported by Force *et al.* (2003). From Fig. 5, it can be seen the reduction in F_V/F_M ratio, a significant fallen in ψ_o=ET_0/TR_0 and substantial increment in DI_0/RC (Fig. 4). Therefore an increase in the effective dissipation of an active RC and the decline in electron transport probability can be used as more accurate parameters to measure photoinhibition than by a decline in F_V/F_M ratio.

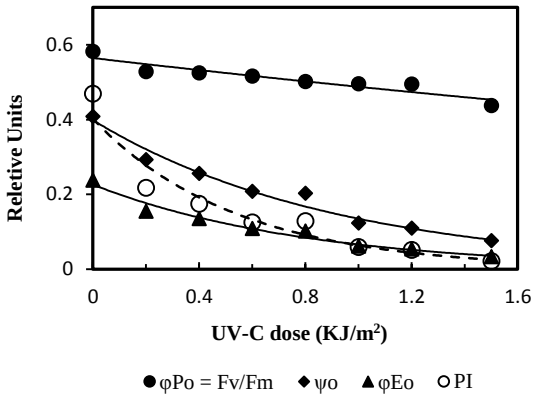

Fig. 5: Flux ratios or yields
Maximum quantum yield of primary photochemistry (φ_{Po}), electron transport efficiency (ψ_o), quantum yield of electron transport (φ_{Eo}), and Performance Index (PI) decreased with increasing UV-C dose.

Among other responses, Paoletti *et al.* (2004), Manes *et al.* (2001) and Nussbaum *et al.* (2001) observed a reduction in φ_{Po} (Eqn. 7). It is further explained, although φ_{Po} is very stable, there can be a reduction when the level of injury is very high under the severe stress conditions. Regarding this, Bussotti *et al.* (2006) suggested, this as a controlled way of absorbed excess light through a down regulation mechanism which includes a decrease of the fraction of fully active RCs and an increase of the fraction of heat sink centers to dissipate excess energy. Thus, the PS II can switch from a process converting light energy into

biochemical energy storage to an energy conversion process that transforms the absorbed light energy into heat dissipation. This mechanism protects the photosystems from the risks of photo-oxidation and allows them to maintain their basal fluorescence intensity (Bussotti *et al.* (2006).

The similar phenomenon was observed by Albert *et al.* (2004), the depression of UV-B stress is more related to stress on the acceptor side (ET_0/TR_0) of PS II than the donor side (TR_0/ABS). Similarly, here also it was pointed out a down regulation by forming non Q_A reducing RCs (heat sink or silent RCs) (Strasser and Micheal 2001, and Strasser *et al.* 2004).

Performance index

PI (Eqn.10) is a good indication about the vitality of the photosynthetic organisms where the decreased vitality as expressed by low PI_{ABS} index values. However, these values were not directly related to the net photosynthesis (Bussotti *et al.*, 2006) since electron flux is not only used in carbon metabolism as it may also be re-routed to other biochemical pathways (Adams and Demming, 2004), such as reduction of oxygen, nitrite, sulfate, or thioredoxin (Bussotti *et al.* 2006).

Fig. 5 shows that the PI as affected by doses of UV-C radiation in comparison to the most commonly used chlorophyll fluorescence parameter, F_v/F_m (φ_{Po}). As was evident, PI which decreased exponentially with increasing doses of UV-C is a highly sensitive index to UV stress, whereas F_v/F_m decreased linearly. The three driving forces of the PI showed the same trend for UV-C sensitivity, though the changes in ψ_o/ (1-ψ_o) are the most pronounced (data not shown). This also indicates that the dark reactions after Q_A- are highly sensitive to UV.

Conclusion

The results of the present study reveal that there are different targets of UV-C radiation on PSII of *Euglena*; photochemistry was less sensitive to UV radiation, biochemistry was the most affected, and non photochemical redox reactions are damaged mainly in the pool. Our results also suggest that the OJIP transients can effectively be used to study stress responses inside the PSII complex.

References

Adams WW and Demming-Adams B (2004) Chlorophyll fluorescence as a tool to monitor plant response to environment. In: Papageorgiou GC and Govindjee (Eds) *Chlorophyll Fluorescence; A Signature of Photosynthesis. Advances in Photosynthesis and Respiration Series (Govindjee, Series Ed.),* Papageorgioupp. Springer, Dordrecht, Netherlands, 583-604.

Aguilera J, Jimenez C, Figueroa FL, Lebert M and Hader DP (1999) Effect of ultraviolet radiation on thallus absorption and photosynthetic pigments in the red alga *Porphyra umbilicalis. J. Photochem. Photobiol. B: Biol.* 48:75-82. DOI: 10.1016/S1011-1344(99)00015-9

Albert KR, Mikkelsen TN and Ro-Poulsen H (2005) Effects of ambient versus reduced UV-B radiation on high arctic *Salix arctica* assessed by measurements and calculations of chlorophyll-a fluorescence parameters from fluorescence transients. *Physiologia Plantarum*, 124: 208–226. DOI: 10.1111/j.1399-3054.2005.00502.x

Bolige A, Kiyota M and Goto K (2006) Circadian rhythms of resistance to UV-C and UV-B radiation in *Euglena* as related to "escape from light" and "resistance to light". *J. Photochem. Photobiol. B.* 81: 43-5. DOI: 10.1016/j.jphotobiol.2005.06.004

Bussotti F, Reto J and Strasser MS (2006) Photosynthetic behavior of woody species under high ozone exposure probed with the JIP-test: A review. *Environmental Pollution*. 147: 430-437. DOI: 10.1016/j.envpol.2006.08.036

Clark AJ, Landolt W, Bucher JB and Strasser RJ, (2000) Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. *Environmental Pollution*. 109: 501-507. DOI: 10.1016/S0269-7491(00)00053-1

Force L, Critchly C and Van Rensen JJS (2003) New fluorescence parameters for monitoring photosynthesis in plants. 1. The effect of illumination on the fluorescence parameters of the JIP-test. *Photosynthesis Research*. 78: 17–33. DOI: 10.1023/A:1026012116709

Govindjee (2004) Chlorophyll a fluorescence: a bit of basics and history. In: Papageorgiou GC and Govindjee (Eds) *Chlorophyll a Fluorescence: A Signature of Photosynthesis, Advances in Photosynthesis and Respiration*. vol. 19, Springer, Dordrecht, Netherlands, 1–41.

Holzinger A and Lutz C, (2005) Algae and UV irradiations: effects on ultrastructure and related metabolic functions. *Micron.* 37: 190-207. DOI: 10.1016/j.micron.2005.10.015

Iriti M, Guarnieri S and Faoro F (2007) Responsiveness of *Lycopersicon pimpinellifolium* to acute UV-C exposure: histocytochemistry of the injury and DNA damage. *Acta Biochemica Polonica*, 54: 273-280.

Kautsky H and Hirsch A (1931) Neue Versuche zur Kohlensaureassimilation. *Naturwissenschaften*.19: 964. DOI: 10.1007/BF01516164

Krause GH and Weiss E (1991) Chlorophyll fluorescence and photosynthesis: the basis. *Ann. Rev. Plant Physio. Plant Molecular Bio.* 42: 313–349. DOI: 10.1146/annurev.pp.42.060191.001525

Kruger GHT, Tsmilli-Michael M and Strasser RJ (1997) Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camelia leaves. *Physiologia Plantarum.* 101: 265–277. DOI: 10.1111/j.1399-3054.1997.tb00996.x

Lazar D (1999) Chlorophyll *a* fluorescence induction. *Biochem. Biophysics*. 1412: 1-28.

Manes F, Donato E and Vitale M (2001) Physiological response of *Pinus halepensis* needles under ozone and water stress conditions. *Physiologia Plantarum*. 113: 249-257. DOI: 10.1034/j.1399-3054.2001.1130213.x

Misra AN, Misra M and Singh R (2012) Chlorophyll Fluorescence in Plant Biology In: Misra AN (Ed) *Biophysics*. ISBN: 978-953-51-0376-9, InTech. DOI: 10.5772/1877

Nussbaum S, Geissmann M, Eggenberg P, Strasser RJ and Fuhrer J, (2001) Ozone sensitivity in herbaceous species as assessed by direct and modulated chlorophyll fluorescence techniques. *J. Plant Physio*. 158: 757-766. DOI: 10.1078/0176-1617-00225

Paoletti E, Bussotti F, Della Rocca G, Lorenzini G, Nali C and Strasser RJ (2004) Fluorescence transient in ozonated Mediterranean shrubs. *Phyton Annales Rei Botanicae*. 44: 121-131.

Prasad SM and Zeeshan M (2004) Effects of UV-B and monocrotophos, singly and in combination, on photosynthetic activity and growth of non-heterocystous cyanobacterium Plectonema boryanum. *Environmental and Experimental Botany*. 52: 175-184. DOI: 10.1016/j.envexpbot.2004.01.013

Strasser RJ (1981) The grouping model of plant photosynthesis: Heterogeneity of photosynthetic units in thylakoids. In: Akoyunoglou, G. (Ed) *Photosynthesis. III. Structure and Molecular Organisation of the Photosynthetic Apparatus.* Balaban International Science Services, Philadelphia, Pennsylvania, 727–737.

Strasser RJ, Tsimilli-Micheal M and Srivastava A (2000) The fluorescence transient as a tool to characterise and screen photosynthetic samples. In: Pathre UA and Mohanty P (Eds) *Yunus Probing Photosynthesis: Mechanisms, Regulation and Adaptation*. Taylor & Francis, London, 445–483.

Strasser BJ and Strasser RJ (1995) Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: *Photosynthesis: from Light to Biosphere,* Mathis P (Ed) Kluwer Academic Publishers, Dordrecht, Netherlands, 977–980. DOI: 10.1007/978-94-009-0173-5_1142

Strasser RJ (1978) The grouping model of plant photosynthesis. In: Akayunoglou G and Argyroudi-Akoyunoglou J (Eds) *Chloroplast Development.* Elsevier/North-Holland Biomedical, Amsterdam, 513–524.

Strasser RJ and Tsimilli-Michael M (2001) Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-Test. Chimie, Nouvelle (SRC).

Strasser RJ, Tsimilli-Micheal M and Srivastava A (2004) Analysis of the chlorophyll-a fluorescence transient. In: Papageogiou GC and Govindjee (Eds) *Advances in Photosynthesis and Respiration*. Springer, Berlin, Germany, 321–362.

Takahashi A, Shibata N, Nishikawa S, Ohnishi K, Ishiokan N and Ohnishi T (2006) UV-B light induces an adaptative response to UV-C exposure via photoreactivation activity in *Euglena gracilis. Photochem. and Photobio. S.* 5: 467-471. DOI: 10.1039/b601971d

Van Heerden PDR, Merope TM, Kruger GHJ, Strasser RJ (2003) Dark chilling effects on soybean genotypes during vegetative development: parallel studies of CO_2 assimilation, chlorophyll-a fluorescence kinetics O-J-I-P and nitrogen fixation. *Physiologia Plantarum*, 17: 476–491. DOI: 10.1034/j.1399-3054.2003.00056.x

Wobeser EAV, Figueroa FL and Cabello-Pasini A (2000) Effect of UV radiation on photoinhibition of marine macrophytes in culture systems. *J. Appl. Phycology*. 12: 2, 159-168. DOI: 10.1023/A:1008198404529