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OJIP fluorescence transient as a tool for analysis of plant responses to insecticides: A review

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ABSTRACT

Fast chlorophyll *a* fluorescence is a useful tool for non destructive analytical use of plant material and relative quantification of plant efficiency under various environmental conditions. The fast fluorescence rise, OJIP, in response to an exposure to a high intensity of saturating pulse (ca 3000 μmol photon/m^2s) is very effective in characterizing the Z-scheme components of the light reaction of photosynthesis in general and of PS II in particular. Various derived parameters, calculated from the peaks of the OJIP transient have been successfully used to analyze the action of biotic and abiotic stresses on the photosynthetic activity of plants. The derived fluorescence parameters precisely determine the site of action of various stressors on non cyclic electron transport chain and the information, so obtained, are applied to minimize the effect of stress on plants as well as to design plants to adapt to such stress situations. The OJIP transient patterns in general and PS II fluorescence in particular are altered by the action of insecticides on plants at field and higher concentrations. Different insecticides have different sites of action on the photosynthetic membrane, which is analysed by the magnitude of changes in various derived fluorescence parameters. This review presents the application of OJIP fluorescence analysis for quick and effective determination of the action of insecticides on photosynthesis.

1. Introduction

Fast Chl *a* fluorescence (2-10 % of the absorbed light; Trissl *et al.,* 1993) has proved to be a very useful and non-invasive tool for plant efficiency studies and more specifically for characterizing the behaviour of photosystem II (PS II; Krause and Weis, 1991; Govindjee, 1995; Lazar, 1999). The chlorophyll fluorescence signal recorded from the plant samples becomes repetitive, non destructive and enables the detection of changes in the response of plants, algae and cyanobacteria to external factors over time. Recent improvements in detecting the fluorescence signal through direct and time-resolved measurements could provide detail information on the fast fluorescence rise. All oxygenic photosynthetic materials investigated so far show a polyphasic rise consisting of the basic steps from the "origin" (O) through two intermittent "inflections" (termed as J and I) to a "peak" (maximum) fluorescence (Kautsky and Hirsch, 1931; Strasser and Govindjee, 1992; Strasser *et al.,* 1995; 2004). The OJIP polyphasic transient was found to change its shape according to changes in the environmental conditions (Munday and Govindjee, 1969; Neubauer and Schreiber, 1987; Krause and Weis, 1991; Strasser *et al.,* 1995).

The analysis of the fast fluorescence rise, termed as OJIP test, allows the derivation of several expressions leading to the actual description of a photosynthetic sample in a current physiological state. Therefore, the changes in the photosynthetic behaviours caused by environmental stress have been widely explored by applying the fast Chl *a*

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fluorescence kinetics in higher plants (Govindjee *et al.,* 1986; Papageorgiou and Govindjee, 2004). The shape of OJIP fluorescence rise has been found to be very sensitive to stress caused by changes (positive or negative) in different environmental conditions, e.g., light intensity, temperature, drought, atmospheric CO_2 concentration and chemical influences (Vernay *et al.*, 2008; Mohapatra *et al.,* 2010; Albert *et al.,* 2011; Bürling *et al.,* 2011; Jena *et al.,* 2012). Various bioenergetic parameters are derived from the OJIP fluorescence data to analyse the site of action of an abiotic or biotic stress on the photosynthetic energy harvesting process (Strasser and Strasser, 1995; Srivastava *et al.,* 1999; Force *et al.,* 2003; Strasser *et al.,* 2004; Tsimilli Michael and Strasser, 2008; Strasser *et al.,* 2010; Yusuf *et al.,* 2010; Stirbet and Govindjee, 2011; Chhotaray *et al.,* 2014).

The analysis of the effects of abiotic stresses on photosynthetic apparatus of green plants (algae, lower and higher plants) and cyanobacteria has been successfully made using the OJIP fluorescence transient and the derived information from the rise (Force *et al.,* 2003; Lazar, 2003; Tsimilli Michael and Strasser, 2008; Vernay *et al.,* 2008; Wu *et al.,* 2008; Strasser *et al.,* 2010; Yusuf *et al.,* 2010; Stirbet and Govindjee, 2011; Jena *et al.,* 2012; Chhotray *et al.,* 2014). There are plenty of information on the analysis of herbicide toxicity to plants and cyanobacteria by the use of fluorescence parameters (DeLorenzo *et al.*, 2001; Juneau and Harrison, 2005; Juneau *et al.*, 2007; Chalifour and Juneau, 2011; Deblois *et al.*, 2013). Some information is also available on the effects of insecticides on photosynthesis through the use of fluorescence signals. However, such information has not yet been compiled to evaluate the potential of fluorescence tools in general and OJIP fluorescence rise in particular as an approach for effective analysis of pesticide phytotoxicity. This review presents the effects of insecticides of various classes on the photosynthetic electron flow by taking the OJIP fluorescence transient. This review is also aimed at scientists, who have some experience with the application of chlorophyll *a* fluorescence but still in the process of discovering its extension to insecticide stress analysis.

2. OJIP parameters for stress analysis

Upon a dark-to-light transition, the fluorescence intensity of a photosynthetic sample increases from a low value (F_0 or O) via two intermediate steps (F_J or J and F_I or I) in 200–400 ms to a maximum value (F_M or P) during the application of a saturating pulse of light (1500-3000 μE/m^2s; Strasser and Govindjee, 1991; Strasser *et al.,* 1995). The different fluorescence rise phases (OJ, JI and IP) can be related to different steps of the reduction of the ETC: OJ parallels the reduction of the acceptor side of PSII (Q_A to Q_B); JI parallels the reduction of the PQ-pool and IP parallels the reduction of the electron transport acceptors in and around PSI (Schansker *et al.,* 2005). This means that OJIP transients give information on the state of the entire non-cyclic ETC. Although complex simulations of OJIP transients use a kinetic model based on the gradual reduction of the ETC (Lazar, 2003; Zhu *et al.,* 2005), it has been shown that the transients can also be approximated assuming that the transients consist of three kinetic components indicating that the rate limitations (exchange of PQ at the Q_B-site of PS II and re-oxidation of PQH_2 by cyt b_6/f) quite effectively separate the three rise phases kinetically. These three phases are found differentially sensitive to chemical stresses (pesticides, herbicides, antibiotics, etc.) and therefore a number of derived parameters are used to determine the electron flow vis-a-vis photosynthetic activity of samples. Table 1 summerises the most commonly used derived parameters of OJIP transient for stress analysis (Force *et al.,* 2003; Strasser *et al.*,2004; 2010; Yusuf *et al.,* 2010; Stirbet and Govindjee, 2011). The trend of change and the magnitude of variation of these parameters are the indications of the variety of effects of stress on plants.

3. Fluorescence rise under insecticide stress

The most significant effect of insecticides on the target pests is the change in the membrane fluidity resulting in various disorders in the insect physiology and the death of the target pest. Similar effects of insecticides on mitochondrial and thylakoid membrane integrity have been reported (Moreno and Madeira, 1990; Mohapatra *et al.,* 1996; Panda *et al.,* 1998; Mohapatra and Schiewer, 2000; Yao *et al.,* 2006; Pan *et al.,* 2008; Li *et al.,* 2010; Chen *et al.,* 2011). Insecticide accumulation in the lipid bilayer core of the thylakoid membrane results in a loose and permeable or a tight and rigid photosynthetic membrane showing abnormal ETC. such effects are easily visualized by the alteration in the chlorophyll fluorescence signal.

3.1 Change in the shape of OJIP fluorescence transient

The shape of the OJIP fluorescence transient, both with short and prolonged illumination (fast and delayed fluorescence) changes in response to insecticide treatments (Singh *et al*., 1996; Pan *et al*., 2008; Li *et al*., 2010; Mohapatra *et al*., 2010; Jena *et al*., 2012). On dimethoate treatment of wheat plants it has been observed that the linear time plots of the fluorescence induction curve of the control as well as treated plants have shown significant variation from each other (Fig. 1). The control plant has a monotonous P to T fluorescence decay whereas treated one has clear S to M fluorescence rise. The fluorescence maximum M became prominent and fluorescence intensity decreased

Table 1

The summary of OJIP parameters, directly obtained from the transient as well as those derived from primary OJIP data. The table is based on the publication by many authors (Strasser and Strasser, 1995; Srivastava *et al.,* 1999; Force *et al.*, 2003; Strasser *et al.*, 2004; Tsimilli Michael and Strasser, 2008; Strasser *et al.,* 2010; Yusuf *et al.*, 2010; Stirbet and Govindjee, 2011).

Sl. No	Parameter	Calculation	Explanation
		OJIP transient data	
1	F_0	50 μs fluorescence	Minimal reliable recorded fluorescence, at 50μs
2	F_t	Fluorescence at time t	Fluorescence at time t after onset of actinic illumination
3	F_K	300 μs fluorescence	Fluorescence at 300 μs
4	F_J	2 ms fluorescence	Fluorescence at the J-step (2 ms) of OJIP
5	F_I	30 ms fluorescence	Fluorescence at the I-step (30 ms) of OJIP
6	F_P (or F_M)	Fluorescence maximum	Maximum fluorescence recorded in the OJIP transient during 1 s
7	F_v	F_M- F_0	Variable fluorescence
8	F_v	$F_t - F_0$	Variable fluorescence recorded at time t after onset of light and t ≠ t_{FM}
9	t_{FM}	Time to achieve F_M.	Time t (in ms) to reach at F_M
10	Area		Total complementary area between fluorescence induction curve and F = F_M
		Energy fluxes	
11	J^{TR} (=TR_0)		Rate of exciton trapping (leading to Q_A reduction) by all PS II RCs
12	J^{ET2} (=ET_0)		Electron transport flux from Q_A to Q_B
13	J^{RE1} (=RE^1)		Electron transport flux until PS I acceptors (defined at t=30ms, corresponding to the I level; also called as End Reaction; RE)
14	J^{DI} (=DI_0)		Rate of energy dissipation in all the PS II
		Quantum yield efficiency	
15	φP_0	$(F_M-F_0)/F_M$	Maximal quantum yield of primary PS II photochemistry
16	φE_0	$(F_M-F_J)/F_M$or $\varphi P_0 .\Psi_0$	Quantum yields of the electron transport flux from Q_A to Q_B
17	φRE^1_0	$(F_M-F_I)/F_M$or $\varphi P_0 .\Psi RE^1_0$	Quantum yield of the electron transport flux until the PS I electron acceptors
18	φD_0	F_0/F_M	Quantum yield (at t = 0) of energy dissipation by PS IIs
19	Ψ_0 or (ΨET^2_0)		1-V_J Efficiency probability with which a PS II trapped electron is transferred from Q_A to Q_B
20	ΨRE^1_0	1-V_I	Efficiency/probability with which a PS II trapped electron is transferred until PS I acceptor
21	δRE^1_0	$(1-V_I)/(1-V_J)$	Efficiency/probability with an electron from Q_B is transferred until PS I acceptors
		Specific energy fluxes (per active PS II RC)	
22	ABS/RC	$(M_0/V_J).(1/\varphi P_0)$	Average absorbed photon per PS II RC (also average antenna size of an active PS II)
23	TR_0/RC	M_0/V_J	Maximum trapped exciton flux per PS II RC (at t=0)
24	ET_0/RC	$(M_0/V_J).\Psi_0$	Electron transport flux (from Q_A to Q_B) per RC (at t=0)
25	DI_0/RC	(ABS/RC)-(TR_0/RC)	Dissipated energy flux per RC (at t=0)
26	RE^1/RC	(M_0/V_J). ΨRE	Electron transport flux until PS I acceptors per PS II RC

		Phenomenological energy fluxes (per excited cross section CS)	
27	ABS/CS_0	F_0	Absorption flux per CS (also apparent PS II antenna size), approximated by F_0
28	ABS/CS_M	F_M	Absorption flux per excited CS, approximated by F_M
29	TR_0/CS_0	$\varphi P_0.F_0$	Trapped energy flux per CS (at t=0)
30	ET_0/CS_0	$\varphi E_0.F_0$	Electron transport flux per CS (at t=0)
31	RE^1/CS_0	$\varphi RE^1.F_0$	Electron transport flux until PS I acceptors per cross section
32	DI_0/CS_0	$\varphi D_0.F_0$	Dissipated energy flux per CS (at t = 0)
		Performance indices	
33	PI_φ	F_v/F_0or $\varphi P_0/(1-\varphi P_0)$	Performance index of primary photochemistry
34	PI_Ψ	$\Psi_0/(1- \Psi_0)$	Performance index of electron transport
35	PI_{ABS}	$PI_\varphi.PI_\Psi$-.(RC/ABS)	Performance index for energy conservation from photons absorbed by PS II antenna to the reduction of Q_B
36	PI^{total}	$PI_{ABS}.[\delta RE/(1-\delta RE)]$	Performance index for energy conservation from photons absorbed by PS II antenna until the reduction of PS I acceptors
37	PI^{total}_{CS0}	$F_0.PI^{total}$	Performance index on cross section basis approximated by F_0
38	PI^{total}_{CSM}	$F_M.PI^{total}$	Performance index on cross section basis approximated by F_M
		Other derived parameters of performance measurement	
39	V_J	$(F_J-F_0)/(F_M-F_0)$	Relative variable fluorescence at J inflection
40	V_I	$(F_I-F_0)/(F_M-F_0)$	Relative variable fluorescence at I inflection
41	M_0	$4(F_K-F_0)/F_M-F_0)$	Net rate of PS II closure by donor limitation
42	S_M	$Area/F_v$	Normalized total complementary area above the OJIP transient (reflecting multiple turnover of Q_A)
43	S_S	V_J/M_0	Normalized total complementary area corresponding only to the O-J phase (reflecting single turnover of Q_A)
44	N	S_M/S_S	Turnover number, i.e. number of Q_A reduction events between time t_0 and t_{FM}
45	V_t	$(F_t-F_0)/(F_M-F_0)$	Relative variable fluorescence at time t
46	ΔV_t	V_t sample-V_t control	Differential relative variable fluorescence transient
47	V_{av}	$1-(S_M/t_{FM})$	Average relative variable fluorescence from time t_0 to t_{FM}
		Density of reaction centres	
48	RC/CS_X	$\varphi P_0.(V_J/M_0).(ABS/CSx)$	Density of RCs per CS
49	RC/ABS	$(V_J/M_0).\varphi P_0$	Active RCs on absorption basis
50	RC/CS_0	$F_0.\varphi P_0.(V_J/M_0)$	Active RCs on cross section basis

slowly to the terminal peak T in dimethoate-treated plants, whereas in the control plants, the fluorescence peak M was not present and fluorescence decreases slowly from S→T (Pandey and Gopal, 2012). This has been attributed to the fluorescence quenching because of the transmembrane ΔpH generated due to reduced across the membrane proton translocation (Briantais *et al.,* 1979, Pandey and Gopal, 2012). Similarly in *Synechocystis* sp. PCC 6803 dimethoate has been reported to cause rapid increase of ΔpH leading to enhanced NPQ resulting in complete loss of variable fluorescence and photosynthetic yield within few minutes (2 – 10 min) of treatment (Mohapatra *et al.,* 1997; Mohapatra and Schiewer, 2000). Further Pandey and Gopal (2012) observed that there are disturbances in the energy redistribution between PS I and PS II (impairment of PS I activity) and/or reduction in the number of mobile light-harvesting protein complex and/or decrease in the activity and concentration of LHC II kinases and/or increase in the activity of LHC II phosphates. Decrease in qP and an increase in NPQ in *P. vulgaris* was found to be the result of

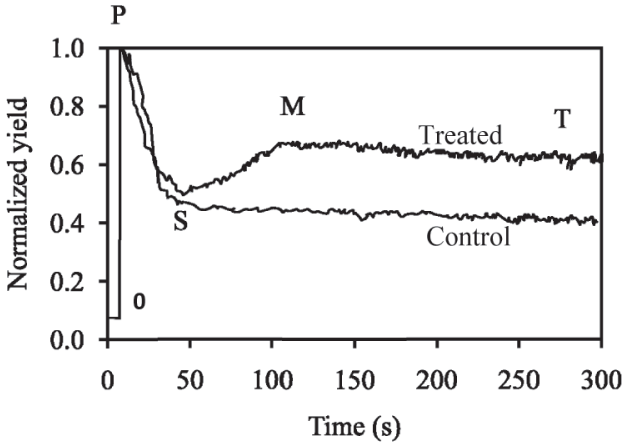

Fig.1. Fluorescence induction curve of control and leaves treated with dimethoate. The values are fluorescence recorded at 685 nm on excitation with 635 nm red diode (Modified from Pandey and Gopal, 2012).

reduced maximum fluorescence associated with SM rise of a leaf beyond 1 sec at weak actinic excitation (Bradbury and Baker, 1984).

In *Solanum melongena* dimethoate treatment, at field concentration, caused an increase of fluorescence at J step, a prolonged IP phase and a reduced P fluorescence, which were proportional to the time up to 6 h but gradual decrease of J fluorescence and restoration of P fluorescence were reported thereafter (Fig. 2; Khillar *et al.,* 2010; Mohapatra *et al.,* 2010). There is also a relative increase of F_0 with time indicating the increase in Q_A reduction. A new step, called K step, first observed and described by Strasser (1997), appears under stress condition when all oxygen evolving complexes (OEC) are in S2 or S3 states and electron donation from Y_Z to $P680^+$ is slowed down (Strasser, 1997; Lazar, 2003; Strasser *et al.,* 2004). Such modification of OJIP fluorescence rise with a distinct and visible K step was, however, not observed with dimethoate treatment as the insecticide inhibited PS II-PS I electron flow but did not cause complete inhibition of PQ-PS I electron flow. The insecticide also did not significantly modify the OEC-PS II electron transport. However, an increase in J amplitude caused a rise in F_0 and indicated the disturbance of electron transport at the acceptor side of PS II (Mohapatra *et al.,* 2010).

Taking four OP insecticides (dimethoate, phorate, quinalphos and chlorfenvinphos) of diverse chemical structures Jena *et al.* (2012) reported that there was alteration in the shape of OJIP fluorescence rise on treatment with each of the four insecticides. Dimethoate and phorate caused enhancement of J fluorescence but reduction of I and P fluorescence (Fig. 3). Higher concentrations of dimethoate, however, reduced OJIP rise at all levels. Quinalphos and

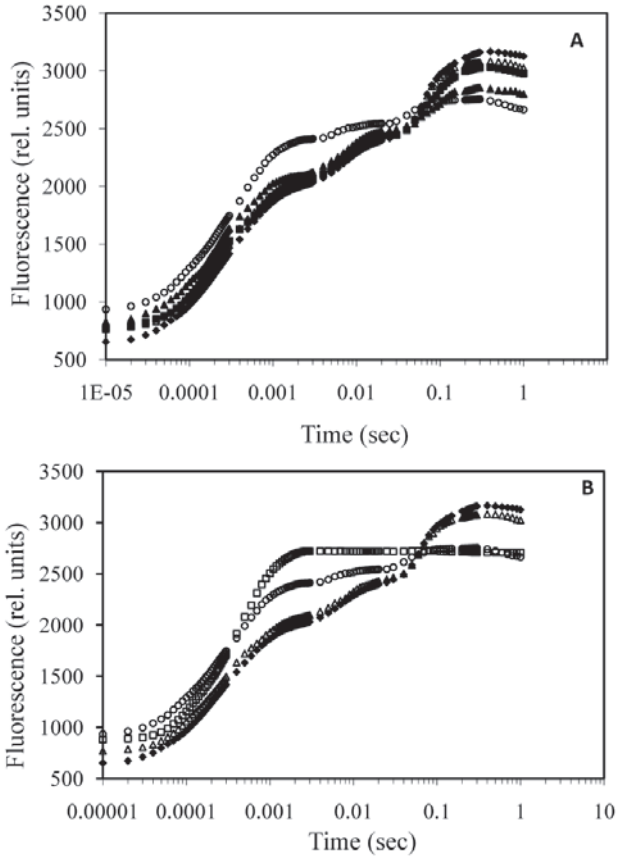

Fig. 2. The OJIP fluorescence rise of dark adopted *Solanum melongena* leaves on treatment with dimethoate (1.419 mg 9.1/ g fr.wt.). (A) Treatment at different time interval (B) Insecticide treatment compared on 0.5 h treatment with DCMU. Treatment time (h): full diamond-0, full square-0.5, full triangle -2, empty circle-6, empty triangle -48, empty square- DCMU (Adapted from Mohapatra *et al.,* 2010).

chlorfenvinphos, on the other hand, limited the energy availability to PS II RC and resulted reduction of OJIP fluorescence rise at all levels. A very fast reduction of F_M with increase in concentration of quinalphos and chlorfenvinphos indicated that there is an inverse relationship between the insecticide concentration and electron flow beyond Q_A due to the donor limitation of PS II – RC (Mohapatra *et al.,* 1997; Mohapatra and Schiewer, 2000). Quinalphos is known to delink the antenna of PS II, most probably by changes in the thickness of the thylakoid membrane in *Synechocystis* (Mohapatra and Schiewer, 2000) and *Chlorella vulgaris* (Jena *et al.*, 2012). Similarly the pyrethroid insecticide cypermethrin also caused significant change in the PS II fluorescence and photosynthetic yield in *Anabaena doliolum* (Mohapatra *et al.,* 2003; Samantaray, 2007) and *Anabaena* sp. PCC 7119 (Chandrakala and Mohapatra, 2012).

Anthracine treatment of *Desmodesmus subspicatus* has shown no significant influence on photochemical processes.

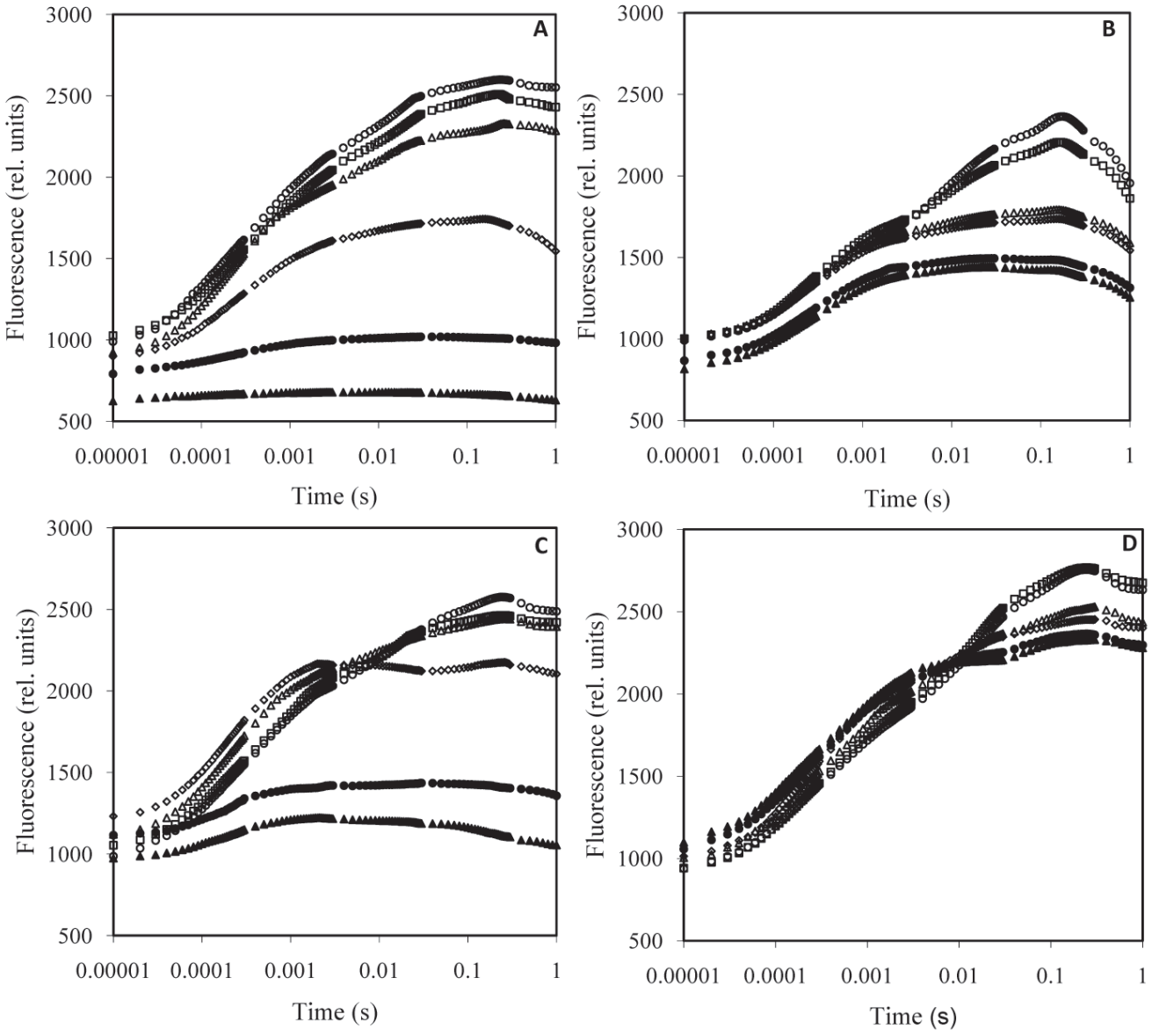

Fig. 3. OJIP fluorescence transients of *Chlorella vulgaris* after one hour treatment with variable concentrations of (A) quanalphos (B) chlorfonvinfos (C) dimethoate (D) phorate Insecticide concentration (μM) : empty circle-0, empty square- 10, empty triangle-50, diamond-100, full circle-200, full triangle-500 (adapted from Jena *et al.,* 2012).

The shape of OJIP fluorescence was also not influenced by the insecticide, an indication of regularised PS II-PS I electron flow (Perales-Vela *et al.,* 2007; Tukaj *et al.,* 2007; Aksmann and Tukaj, 2008; Kummerova *et al.,* 2008). On the other hand, high concentrations of artemicinin treatment of *M. aeruginosa* caused disappearance of fluorescence at P level and the JIP phase gradually levelled off with increasing artemisinin concentration into a OJ rise (Ni *et al*., 2012). This suggested that artemisinin blocked the electron transfer beyond Q_A, like that of a standard herbicide (Srivastava *et al*., 1998; Ni *et al.*, 2012).

Acetamiprid treatment of *Synechocystis* caused a concentration-dependent change on the OJIP curve decreasing almost in parallel with increasing acetamiprid concentration (Li *et al.*, 2010). Fluorescence rise was slow from J step to I step, a typical shape of the rise in cyanobacteria, for the control and low concentration acetamiprid stressed cells. However, at high concentrations (≥ 1 mM), the J–I phase rise almost disappeared (Fig. 4).

3.2. Effects on plant efficiency and energy dissipation

In most of the cases insecticide treatment induces the rise of stress indicating parameters viz., V_J, M_0, DI_0/RC and TR_0/RC (Mohapatra *et al*., 2010; Khillar *et al*., 2010; Li *et al*., 2010, Jena *et al*., 2012). Force *et al.* (2003) noted that TR_0/RC showed narrowest magnitude of change under any stress situation and did not throw much light on the adaptive

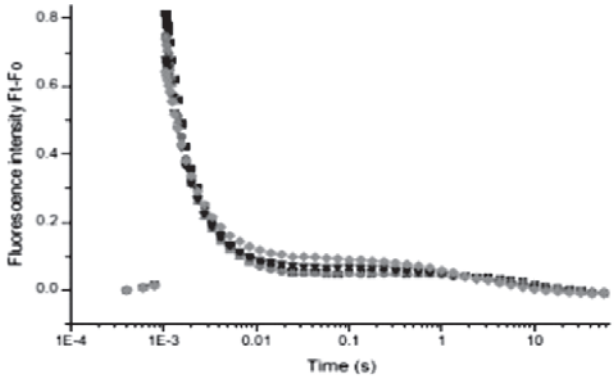

Fig. 4. The Q_A^- reoxidation kinetics of *Synechocystis sp.* treated for 24 h with various concentrations of acetamiprid. Insecticide concentration (μM): square-0, circle-50, triangle-100, diamond-500 (Li *et al*., 2010).

response of plants. Many other fluorescence parameters, viz., F_v, TR_0/Abs, ET_0/TR_0 and ET_0/RC, are adversely affected by the insecticide due to suppression of electron transport in functional PS II (Bukhov *et al*., 2004; Volgusheva *et al*., 2008; Bascik-Remisiewicz *et al*., 2011; Ni *et al.,* 2012; Jena *et al*., 2012; Antal *et al*., 2013). Thus the insecticide not only minimizes the functional PS II but also retards the electron transport. These parameters are affected with reduction in the fluorescence yield due to the adverse effects of the insecticides on donor, acceptor or both sides of PS II.

Decrease in the value of φP_0, Ψ_0 and φE_0 on treatment with the insecticides indicated the slowing down of electron flow from PS II to PS I and a corresponding increase of V_J and M_0 proved that the limitation of Q_A^- reoxidation is due to poor diffusion of PQ across the thylakoid membrane (Jena *et al.* 2012). Reduction in PI_{total} with insecticides treatment was caused due to acceptor limitation of PS I (Mohapatra *et al.,* 1997). V_J and M_0 increased gradually with artemisinin concentration, and there was proportionate decrease of electron transport beyond Q_A. The increased V_J and decreased ΨE_0 indicate reduced Q_A accumulation as observed under light stress conditions (Ni *et al*., 2012).

Anthracine treatment also caused decrease in the amount of energy absorbed (ABS/RC) and trapped (TR_0/RC) by active RC suggesting greater antenna size and photosynthetic activity of single RC in anthracine-treated cells. At the same time, the fraction of active PS II RCs was a little lower than in controls as some RCs are converted into heat sinks when over-excitation of photosynthetic pigments occurs. These RCs become inactive, i.e., they are unable to reduce Q_A (Krause and Weis 1991), and this kind of NPQ may be correlated with the rearrangement of the PS II core or with degradation of PS II proteins (Aksmann and Tukaj, 2008). However, anthracine diminishes the performance indices of photosynthetic cells by reduction of the fraction of active RCs and from the lower quantum efficiency of energy trapping (φP_0) and electron transport (φE_0) (Huang *et al.,* 1997; Aksmann and Tukaj, 2008; Bascik-Remisiewicz *et al.,* 2011).

The parameter S_m is a measure of the energy needed to close all RCs and shows the multiple turnover. The value of S_m and N could reflect the size of PQ pools. If an exponential fluorescene rise for the single turn-over situation, then the normalized area S_s would be inversely proportional to the initial slope of the relative variable fluorescence (Strasser *et al.* 1995). However, the value of this slope can be calculated utilizing only data from the *in vivo* fluorescence transient as M_0 without the requirement of additional measurements in the presence of DCMU (Strasser *et al.,* 1995; Srivastava *et al.,* 1998; Strasser *et al.,* 2004). Artemisinin stress had little effects on the minimum value of S_m (S_s) but had greater impact on the rate of oxidation and reduction of Q_A (M_0), S_m and N. This confirmed that the acceptor side in the electron transport of PS II was a target site of artemisinine stress in *M. Aeruginosa* (Ni *et al.,* 2012). Similar acceptor limitation of PS II has been reported in algae and cyanobacteria (Mohapatra *et al.,* 1997; Li *et al.,* 2010; Jena *et al.,* 2012).

The rise in S_s with insecticide treatment has also been reported by many workers. This shows that Q_A reduction rate increases, but the energy of reducing a single Q_A remains unchanged. On the other hand, since S_m decreases, i.e. PQ storage capacity of PS II reaction centre receptor side decreases, the corresponding redox number of Q_A also reduces (Lazar, 2003; Strasser *et al.,* 2004; Li *et al.,* 2010; Mohapatra *et al.,* 2010; Jena *et al.*, 2012).

Corresponding to the insecticide concentration, φP_0 of *Synechocystis sp.* was not, however, significantly affected after exposure to acetamiprid, which indicated that this parameter of the transient is not a good indicator of stress response (Li *et al.,* 2010). However, with OP insecticide treatment, significant reduction in the magnitude of φP_0 has been reported (Mohapatra *et al.,* 1997; 2010; Mohapatra and Schiewer, 2000; Pandey and Gopal, 2012; Jena *et al.,* 2012). Further Mohapatra *et al.* (2003) and Samantaray (2007) observed that with pyrethroid insecticide treatment most of the performance indicating parameters including φP_0 of algae and cyanobacteria are adversely affected.

M_0, ABS/RC, TR_0/RC and φD_0 changed little under stress of various concentrations of acetamiprid, indicating that acetamiprid had little effect on energy flux per RC. φE_0, performance index (PI_{CS} and PI_{ABS}) were promoted by

lower concentration (0.1 mM and below) acetamiprid but remarkably reduced by higher concentrations (Li *et al.,* 2010*)*. On the other hand, energy dissipation and the dissipation flux per active RC significantly decreased with other insecticide treatments (Mohapatra *et al.,* 1997; 2010; Mohapatra and Schiewer, 2000; Pandey and Gopal, 2012; Jena *et al.,* 2012). However, on the basis of cross section, the active RC/CS_0, ABS/CS, TR_0/CS, ET_0/CS clearly decreased with increasing acetamiprid concentration. (Table 2). All these observations proved that Q_A reoxidation kinetics was not significantly affected by lower concentrations (0.1 mM or below) of acetamiprid but at higher concentrations the effect was significant. Acetamiprid decreased the density of active PS II RC per excited cross section and quantum yield of electron transport, resulting in the decline of performance of PS II (Li *et al.,* 2010).

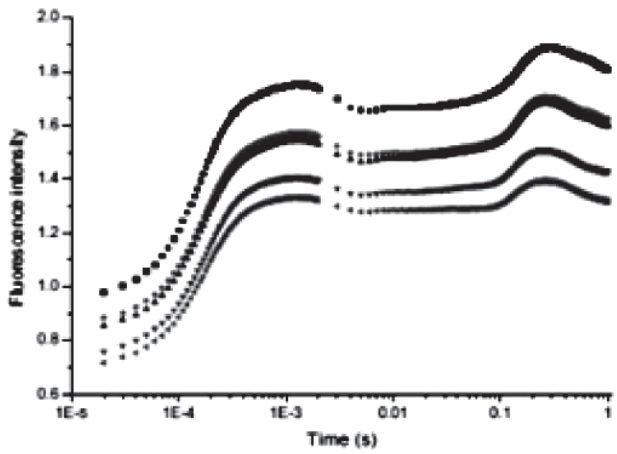

Fig. 5.The fluorescence transient of *Synechocystis* sp. untreated and treated for 24 h with various concentrations of acetamiprid. Legend for concentrations are as for figure (Li *et al.,* 2010).

Table 2

The JIP-test parameters of *Synechocystis s*p. cells cultured in various concentrations of acetamiprid for 24 h. All parameters were normalized to the control (Li *et al.,* 2010)

Conc (mM)	V_J	M_0	F_v/F_m	ψ_0	φE_0	φD_0	ABS/RC	TR_0/RC	ET_0/RC	RC/CS_0	ET_0/CS	PI_{cs}	PI_{abs}
0	1	1	1	1	1	1	1	1	1	1	1	1	1
0.5	0.992	0.966	0.998	1.036	1.034	1.002	0.976	0.974	1.009	0.925	0.935	0.962	1.064
0.1	0.978	0.962	1.024	1.104	1.129	0.977	0.961	0.984	1.087	0.907	0.987	1.069	0.978
0.5	1.022	0.994	1.03	0.894	0.92	0.972	0.944	0.972	0.87	0.821	0.715	0.762	0.978
1.0	1.074	1.038	1.007	0.652	0.657	0.993	0.959	0.967	0.631	0.763	0.481	0.469	0.639

The amplitude of the fast phase and the middle phase rise slightly, accompanied with a slight drop of the amplitude of the slow phase at low concentrations of acetamiprid (Li *et al.,* 2010). This means that low concentrations of acetamiprid may stimulate electron transport from Q_A to Q_B. The disappearance of this J–I phase rise at 1.0 mM acetamiprid implies that the reduction Q_B, PQ, Cyt and PC may be inhibited by high concentration of acetamiprid. Besides, the effect on J–I phase might also be related to the membrane potential changes that may also affect the J–I phase (Fig. 5).

4. Conclusion

The fast OJIP fluorescence transients and their quantification by OJIP test provide a rapid, reliable and non-invasive tool to detect real time changes in the functionality of the photosynthetic apparatus and plant vitality in physiologically active or stress conditions. The OJIP transient pattern as well as the analytical parameters have been successfully used to evaluate the plant performances as well as to predict the production on the basis of plant performance. The behaviour of photosystems could be effectively analyzed by OJIP fluorescence measurement making it a valuable and potential tool for plant efficiency analysis. New analytical parameters are being adopted to the OJIP fluorescence interpretation, which are expected to add new dimension to the effectiveness of this analytical method. These parameters would possibly help in quick detection of various stresses, inclusive insecticides, on crop plants and may be of use in regulating the pesticide use to minimize residual toxic effects.

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