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BRIEF COMMUNICATION

Dimethoate-induced slow S to M chlorophyll *a* fluorescence transient in wheat plants

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Abstract

In eukaryotic oxygenic photosynthetic organisms (both plants and algae), the maximum fluorescence is at peak P, with peak M lying much lower, or being even absent. Thus, the PSMT phase, where S is semisteady state, and T is terminal state, is replaced by a monotonous P→T fluorescence decay. In the present study, we found that dimethoate-treated wheat plant leaves showed SM transient, whereas in the case of control plants monotonous P→T fluorescence decay occured. We suggest that this was partly due to quenching of fluorescence due to [H^+], responsible for P to S (T) decay in control plants (Briantais *et al*. 1979) being replaced by state transition (state 2 to state 1) in dimethoate-treated plants (Kaňa *et al*. 2012).

Additional key words: chlorophyll *a* fluorescence; dimethoate; Kautsky transient; slow fluorescence transient; state transition; wheat.

Introduction

The change of chlorophyll (Chl) *a* fluorescence intensity that occurs when a photosynthetic sample, kept in darkness, is exposed to light is known as Chl fluorescence induction (FI) or the Kautsky transient. Upon illumination, a dark-adapted plant leaf undergoes a fast fluorescence rise from a minimum level O to a maximum level P within 500 ms and then it slowly decreases to a steady state terminal fluorescence level T within 3–5 min or more (Kautsky and Hirsch 1931) (for reviews, *see* Govindjee and Papageorgiou 1971, Papageorgiou 1975, Govindjee 1995, Stirbet and Govindjee 2011, Papageorgiou and Govindjee 2011).

Fluorescence induction kinetics can be distinguished in the three phases: (*1*) a fast OJIP rise, where J and I are inflections; (*2*) a slow P to S decay, and (*3*) a much slower S to M to T phase. O(JI)P transient is completed within a second (up to 500 ms) (Lazár 1999, Pandey and Gopal 2011a), the slow PS decay may take a few seconds and the S(M)T transient is completed in few (3–5) min (Papageorgiou *et al*. 2007). The rising portion of the curve (the O to P rise) reflects the primary photochemical events of photosystem (PS) II of photosynthesis (Strasser *et al*. 1995, Stirbet and Govindjee 2011). On the other hand, the P to S phase is related to several factors that include reoxidation of the first plastoquinone electron acceptors of PSII, Q_A, by PSI enabling thus a photochemical fluorescence quenching, (q_P), the energization of the thylakoid membrane (q_E) due to the transmembrane ΔpH), photoinhibition (q_I) and a contribution of state 1 → 2 transition (Briantais *et al*. 1979) nonphotochemical fluorescence quenching (NPQ). The SMT phase of FI has been suggested to be due to state transitions

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Abbreviations: Chl – chlorophyll; FI – fluorescence induction; LHCII – light-harvesting complex II; NPQ – nonphotochemical quenching of Chl fluorescence; OJIPSMT – names for the various points in Chl fluorescence transient; PAR – photosynthetically active radiation; PQ – plastoquinone; PS – photosystem; Q_A – primary quinone electron acceptor of PSII; Q_B – secondary plastoquinone two-electron acceptor of PSII; q_P – photochemical quenching of Chl fluorescence; ΔpH – transthylakoid proton gradient.
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(redistribution of light energy between PSI and PSII); the SM rise being due to a transition from low fluorescent state 2 to higher fluorescent state 1. Further, redistribution of light energy into PSI, in state 2, may be initiated by phosphorylation of the light-harvesting Chl proteins after the plastoquinone (PQ) pool is reduced (Papageorgiou *et al*. 2007, Papageorgiou and Govindjee 2011).

In dark-adapted plant leaves, the primary quinone acceptor (Q_A) and the secondary quinone acceptor (Q_B) are maximally oxidized. The reaction centers of PSII are "open". The fluorescence is minimum at the O level. Upon illumination, Q_A becomes reduced and transfers electrons to Q_B. During the rise up to the P level, the PQ pool becomes fully reduced and by that time all the electron acceptors beyond PSI are also reduced (Munday and Govindjee 1969). At the point of maximum fluorescence (P level) the Q_A, Q_B, and the PQ pool are maximally reduced (Lazár 2006). The reaction centers of PSII are then "closed". With the onset of PSI activity, which reoxidizes the PQ pool as well as Q_A^- and $Q_B^{-,-}$, as well as several other processes, the maximum fluorescence slowly decreases to its terminal steady state T. At the terminal fluorescence level T, the photosynthetic apparatus is in state 2 (Strasser *et al*. 1995, Baker 2008, Lichtenthaler and Rinderle 1988, Papageorgiou and Govindjee 2011). state transition (state 1 → 2) has been suggested to be regulated by the light-dependent phosphorylation of the light-harvesting Chl protein complex of PSII (LHCII). The redox state of the plastoquinone regulates the activity of protein kinase that phosphorylates LHCII, and the binding of the plastoquinol at the Q_0 site of the cytochrome b_6f complex is responsible for the activation of these protein kinases. After phosphorylation, this mobile light-harvesting protein complex detaches from PSII and attaches to PSI. On the other hand, the reverse, state 2 to state 1, is initiated by dephosphorylation by a phosphatase, of the mobile light-harvesting Chl protein complex, that was attached to PSI, to move from PSI to PSII serving to collect light for PSII. In this way photosynthetic apparatus controls state 1 ↔ 2 transitions (Allen 2003, Murata 2009, Papageorgiou and Govindjee 2011).

Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) is a foliar applied systemic thio-organophosphorus insecticide; it is used in various countries to control the insect population on a wide variety of crops. Dimethoate produces its insecticidal effect through inhibition of acetylcholinesterase, an enzyme that terminates the action of acetylcholine by catalyzing its hydrolysis, thereby disrupting the normal functioning of nerve transmission, producing hyper-excitability, convulsion, muscular paralysis, and respiratory failure (O'Brien 1967). Studies on the phytotoxic effects of organophosphorus insecticides on phytoplankton have suggested that this type of compound reduces growth rates and inhibits the synthesis of Chl, proteins, and carbohydrates (Mohapatra and Mohanty 1992, Chen *et al*. 2007, Pandey and Gopal 2011b). In addition, dimethoate is known to induce enhancement of respiration of lake phytoplankton community (Chen *et al*. 2007, Piska and Waghray 1991). Dimethoate could also affect plasma membrane, PSII activity and photophosphorylation of *Synechocystis* cells and cause inhibition of photosynthetic electron transport (Mohapatra *et al*. 1997, Mohapatra and Schiewer 1998). It was also shown that dimethoate negatively affects electron transport between PSII and PSI at a concentration of 2 mM (Chen *et al*. 2007); with increasing concentration, it also causes production and accumulation of reactive oxygen species (ROS) and it increased activity of antioxidants such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Mishra *et al.* 2009). Dimethoate accumulates in plant leaves (Lucier 1967), which are the major sites of metabolic activities and it inhibits plant growth. The inhibition of morphological parts may be associated with changes in cell division and cell elongation (Tevini and Teramura 1989); there is also the possibility of conversion of indole-3-acetic acid (IAA) into various photooxidative products (Ros and Tevini 1995), and production of excess ROS.

Wheat plant shows monotonous PT fluorescence decay. In the present study, we showed that treatment of wheat plants with dimethoate led to a clear observation of the SM fluorescence rise; this novel observation requires future experimentation. However, we suggest that this was partly due to quenching of fluorescence due to $[H^+]$, responsible for P to S (T) decay in control plants (Briantais *et al*. 1979) being replaced by state transition (state 2 to state 1) in dimethoate-treated plants (*see* Kaňa *et al*. 2012 for the interpretation of SM rise in cyanobacteria).

Materials and methods

Plant growth and treatment with the insecticide: Healthy and uniformly sized seeds of *Triticum aestivum* L. (var. PBW 343) were surface-sterilized in 4% sodium hypochlorite solution (v/v, in double distilled water) for 20 min and presoaked for 20 h in distilled water and wrapped in wet cloth overnight. Selected uniformly germinated seeds were transferred into small plastic pots containing acid-washed sterilized sand (≈ 260–270 g). Each pot contained 4 wheat plantlets. Plants were grown in a growth chamber under illumination of 90 μmol (photon) m^{-2} s^{-1} photosynthetic active radiation (PAR) obtained from four fluorescent tubes (*Philips*, India) and at 23 ± 2°C in a 14-h photoperiod with relative humidity of 50%. After 3 d of germination, plants were irrigated with 0.2% modified Rorison medium [0.4 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.2 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM KH_2PO_4,

0.1 μM $CuSO_4 \cdot 5H_2O$, 0.2 μM $ZnSO_4 \cdot 7H_2O$, 9.2 μM H_3BO_3, 1.8 μM $MnCl_2 \cdot 4H_2O$, 0.2 μM $Na_2MoO_4 \cdot 2H_2O$ and 10 μM Fe-EDTA]. The dimethoate (800 ppm) was given to the plant roots along with nutrient medium (in 20 ml) on alternate days and the first treatment was given after 6 d of germination. To analyze the effect of dimethoate, plant leaves were used after 10 d of the first treatment. At the time of analysis, plants were 16 d old with 4–5 leaves and about 16–17 cm high.

Fluorescence induction, excited by 635 nm red diode laser: Fluorescence induction curves of 20 min pre-darkened plant leaves were recorded using a computer-controlled *Acton 0.5* meter triple grating monochromator (*Acton Research Corporation*, USA**)**, and *Hamamastu R928* photomultiplier (*Hamamatsu Photonics*, Japan**)** as a detector. The sample was excited by a red diode laser light (635 nm, 10 mW). The lower abaxial side of all the 4 to 5 leaves of each plant were stacked in parallel on a black wood cardboard. The leaf fluorescence was excited and measured at an angle of 45º to the leaf plane. The beam expander was aligned to obtain 2 cm^2 expanded laser light beam. The shape of the light beam on the sample was elliptical. The intensity of laser light was 265 μmol(photon) m^{-2} s^{-1}. Chl *a* fluorescence was collected at the entrance slit of the monochromator. The fluorescence intensity was recorded as a function of time (up to 5 min) at 685 nm, using intensity *vs.* time mode of *Spectra Sense* software (*Roper Scientific Acton Research*, USA**)**. The time resolution of fluorescence detection was 500 ms. Fluorescence points of the FI curve (P, S, M, and T) were determined from these curves.

Results and discussion

The linear time plots of the FI curve of the control as well as dimethoate treated wheat plants are shown in Figs. 1 and 2. The control plant showed monotonous P to T fluorescence decay; whereas dimethoate (800 ppm)-treated wheat plant leaves showed clear S to M fluorescence rise. The fluorescence maximum M became prominent and fluorescence intensity decreased 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.

The SM transient was clearly present in the dimethoate-treated wheat plant leaves. The decrease, the P to T decay, suggested that state transition (state 1 →2) might be taking place; however, superimposed on it was the fluorescence quenching because of the trans-membrane ΔpH, $q_{\Delta pH}$ due to proton translocation (Briantais *et al*. 1979). Increase in the SM transient might suggest the opposite, *i.e*., state 2 to state 1 transition (Kaňa *et al*. 2012); further there might be disturbance in the energy redistribution between PSI and PSII (impairment of PSI activity) and/or reduction in the number of mobile light-harvesting protein complex and/or decrease in the activity and concentration of LHCII kinases and/or increase in the activity of LHCII phosphates.

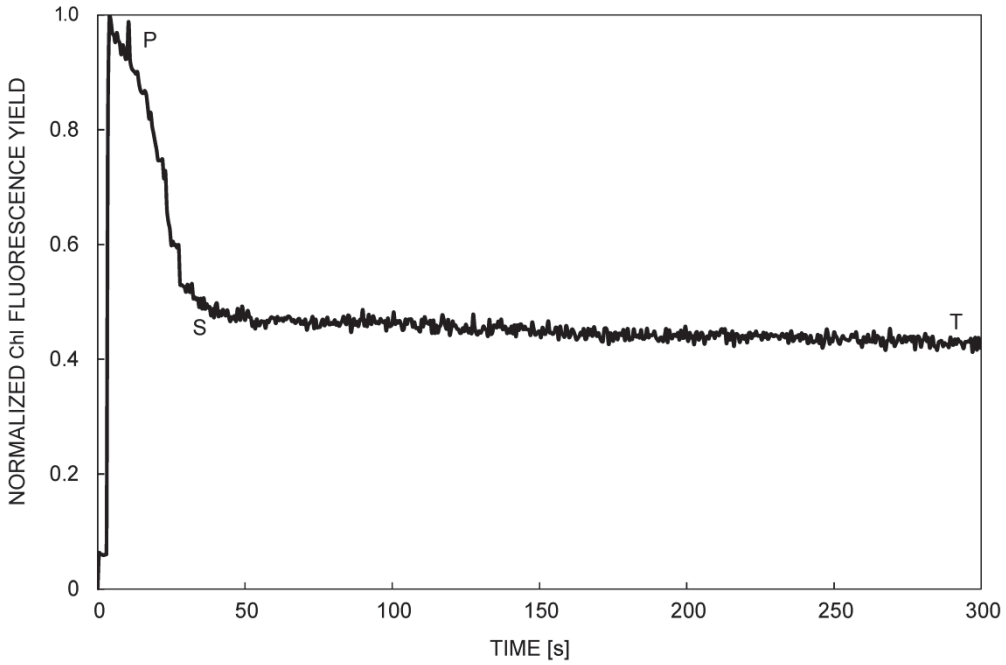

Fig. 1. Monotonous P→T fluorescence-induction curve of wheat leaves without dimethoate treatment, at the wavelength of 685 nm, excited by 635 nm red diode laser.

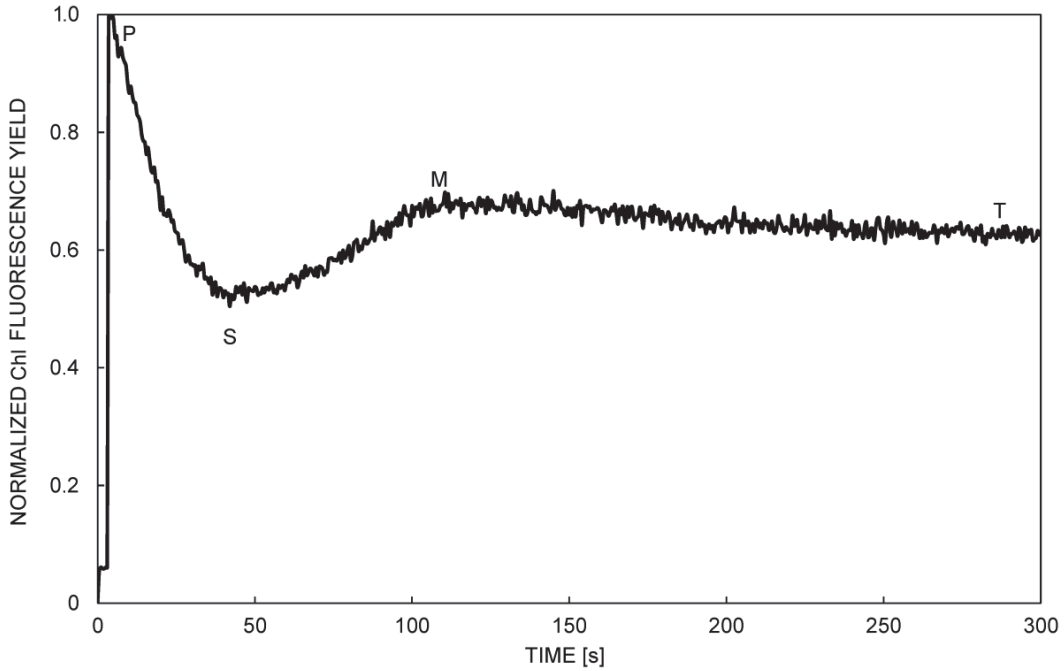

Fig. 2. Fluorescence-induction curve of wheat leaves treated with dimethoate, at the wavelength 685 nm, excited by 635 nm red diode laser showing SM transient.

The SM rise due to dimethoate treatment may also be due to a decrease in the q_P since Bradbury and Baker (1981, 1984) suggested that SM rise of a *P. vulgaris* leaf, at weak actinic excitation, corresponds predominantly to a decrease in q_P and an increase in NPQ. This makes no sense as NPQ would reduce fluorescence, not to increase it. Using light scattering ($\triangle A_{535}$) and 9-aminoacridine fluorescence, Sivak *et al.* (1985a and 1985b) and Horton (1983) observed a decrease in the NPQ. The involvement of q_P in the SM rise in the higher plant leaves is supported also by parallel rise in the rate of O_2 evolution (Papageorgiou and Govindjee 1968a, 1968b) and of CO_2 fixation (Ireland *et al.* 1984, Walker *et al.* 1983). We conclude that the slow S to M Chl *a* fluorescence transient in the treated plants is due to the state transition (state 2 to state 1) induced by dimethoate. It replaced the quenching of fluorescence due to $[H^+]$, responsible for P to S (T) decay in control plants, is currently the most logical explanation. Further experiments are needed to discover the actual mechanism of the phenomenon.

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