

## RESEARCH ARTICLE

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## Effect of Light Intensity on Chlorophyll Fluorescence in Olive Plants – Application of mPEA Fluorometer.

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### Abstract

Light is one of the basic ecological physics factors, determining not only the possibility for plants existence but also the physiological state of their photosynthetic apparatus. Applying simultaneous measurements of the kinetics of prompt fluorescence, delayed fluorescence and modulated 820 nm reflection we investigated the response of the photosynthetic apparatus when changing the light intensities ( $300 - 4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) on olive plant leaves. We describe the experimental approaches to studying the state of the photosynthetic apparatus and ways to study important structural and functional parameters, such as the quantum efficiency of the electronic flow in the Photo System II, the Photo System I and the electron transport chain between the two photo-systems; the concentration of the active reaction centers of Photo System II; the electronic capacity of the electronic transport chain; as well the total parameter that characterizes the productivity in photosynthetic apparatus initial reactions.

**Keywords:** photosynthesis, chlorophyll fluorescence, prompt fluorescence, delayed fluorescence, JIP test, light intensity.

### 1. Introduction

In our approach, the derivation of links between the energetic behavior of the photosynthetic apparatus and the fluorescence signals is based on the ‘Theory of Energy Fluxes in Biomembranes’ [26,27]. The theory was formulated in a general way, so that it could be applied to any pigment assembly in any type of biomembrane. It has been mainly applied to studies in higher plants and algae, on energy exchange within and between Photo System II (PhS) units, as well as from PhS II to PhS I.

The methodological framework of the energy flux theory permits the rigorous definition of all terms often used in the analysis of energy distribution in the photosynthetic apparatus (flux, yield, probability and rate constants of excitation energy transfer). The theory introduces and defines five basic and distinct quantities related to any pigment system (see scheme Figure 1 and [29] and postulates that, for any possible complex arrangement of interconnected pigment systems, their energetic communication can be expressed by simple equations, easily solved analytically, in terms of the five basic quantities.

Simultaneous chlorophyll (Chl) a fluorescence and 820 nm transmission measurements have provided experimental evidence that the three phases (i.e. O-J, J-I and I-P) of the prompt fluorescence rise OJIP [13] reflect three different reduction processes of the electron transport chain [28]. Following a dark-to-light transition of a photosynthetic sample, prompt fluorescence (PF) is emitted and during light-to-dark transition, delayed fluorescence (DF) emission is detected. DF was discovered by Strehler and Arnold. It is mainly emitted from PhS II, and PhS I contributes very little to the DF emission. PF depends on the redox state of the PhS II reaction centers (RC); however the DF in a time range from several microseconds to milliseconds, after light excitation, reflect the recombination, in the dark, between the reduced primary electron acceptor  $\text{QA}^-$  and the oxidized donor ( $\text{P680}^+$ ) of PhS II that are formed after light-induced charge separation [29]. DF has components that decay in very different time domains.

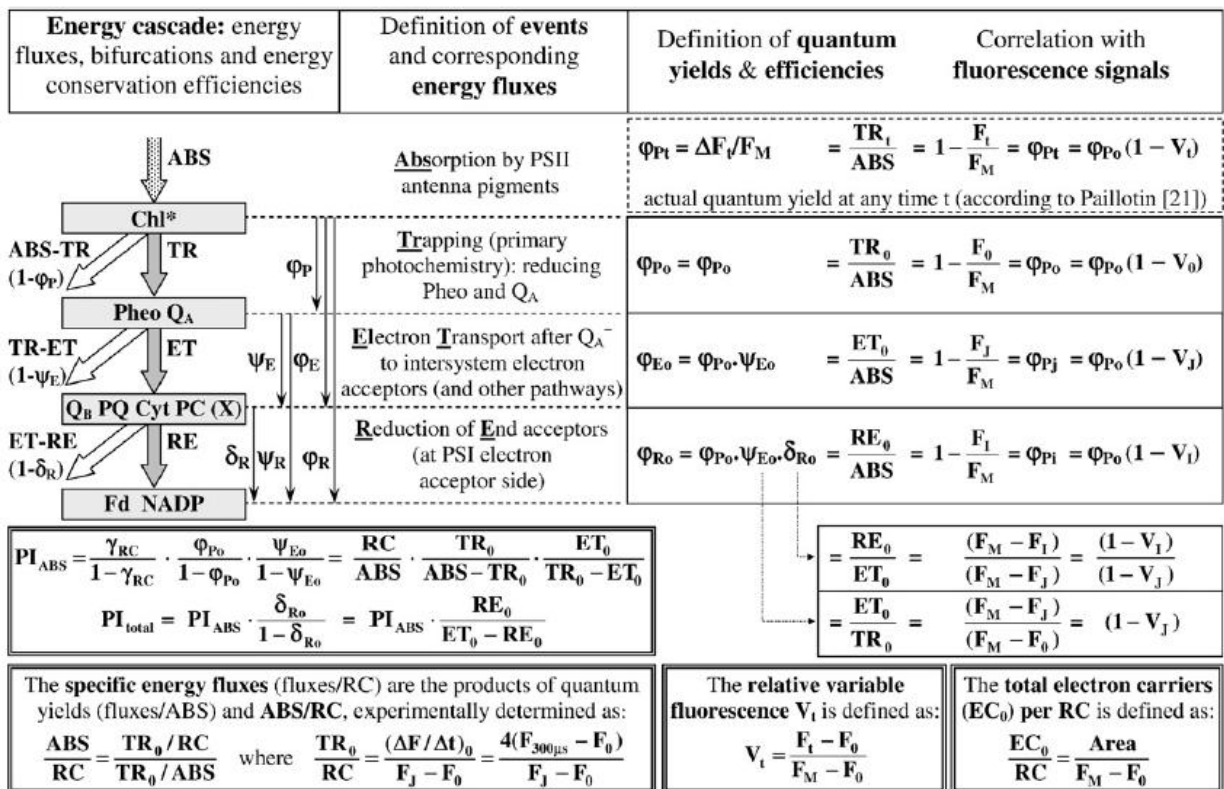
It is considered that the emission spectra of the prompt chlorophyll fluorescence and delayed fluorescence emission are essentially identical. The intensity of DF depends directly on the rate of

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backward electron transport reactions in the RC of PhS II. The shape of the DF induction curve depends on the sample type and its physiological state; further, DF induction curve depends on the kinetic components of DF being measured.

It is known that if the light intensity absorbed by the photosynthetic pigments is too high, all the absorbed energy can not be used in the process of photosynthesis. When the absorption rate of light quanta significantly exceeds the possibility of using their energy for photosynthesis, a condition called light stress occurs in plants. As a result of light stress,

functional disturbances of photosynthetic reactions (photo inhibition) and even damage to the photosynthetic apparatus can occur [20]. There are many mechanisms for protecting the reactions of the light phase of photosynthesis and the structure of the thylakoids (mainly the PhS II complex). Plants can protect themselves from light stress and photoinhibition and thus avoid damage caused by excess radiation [18], but may also prevent excessive phase, or restore [1,2,3,14]. Numerous studies showing that PhS II plays an important role in protecting the photosynthetic apparatus [4,25].



**Figure 1:** A schematic presentation of the JIP-test (by Reto J. Strasser ). For details see Material and methods section.

In this study light intensities effects in olive plants was obtained in vivo, using three signals- PF, DF and modulated reflection (MR) 820 nm, measured simultaneously. We have shown that simultaneous measurement of PF, DF and MR is an important tool to characterize the effect of actinic light intensity on photosynthetic systems and can be used as a tool to monitor these changes induced in the photosynthetic membranes.

## 2. Material and Methods

### Plant material

In this paper we studied olive seedlings grown in the conditions of natural solar lighting in Albania. It is known that growing in bright light helps plants

adapt to excess light energy and develop different acclimatization adaptation mechanisms that serves mainly to protect the photosynthetic apparatus.

Olea europea L. sativa, “Kalinjot” and “Ulliri i holle i Himares” seedlings, autochthonous cultivars, grown in nature, were collected from the Center for Agricultural Technologies Transfer, Vlore and transferred to the laboratorys of Department of Biophysics, Faculty of Biology, University of Sofia “Kliment Ohridski”, where they continued to grow stored in air temperature 22-25 oC, day/night mode 12: 12h and luminescent lighting with intensity 250  $\mu\text{mol h} \cdot \text{m}^{-2}\text{s}^{-1}$ .

The Multifunctional Plant Efficiency Analyser m-PEA

Experiments were carried out with mPEA fluorimeter in vivo on unbroken leaves. All measurements were conducted on dark-adapted for 1 h plants. Working head held on a flexible tripod, is placed over the upper surface of the selected leaf and the photosynthetic parameters, JIP transients are recorded. Depending on the experiments purpose, using the m-PEA Protocol Editor, the operator configures preliminarily the measurements protocol. The system is controlled from a comprehensive Windows® software package (m-PEA<sup>+</sup>) which allows complex experiments to be designed, uploaded and executed by the m-PEA hardware. Recorded data is quickly downloaded to the software via a USB2.0 connection.

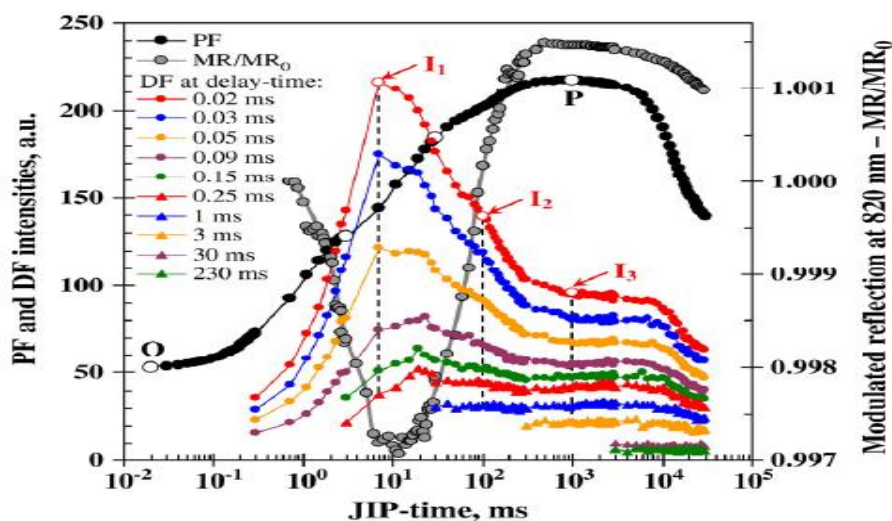
In the m-PEA instrument emitter wavelength ranges are: 627610 nm, for the actinic light LED; 820625 nm, for the modulated light LED, and 735615 nm, for the far-red light LED; the latter uses a RG9 long pass filter to remove any visible light component [12,28]. The actinic light LED is built into the centre of the optical sensor unit and focused into the sample surface to provide homogeneous illumination over the exposed circular area (2 mm diameter), with an intensity of  $5000 \mu\text{mol h}^{-1} \text{m}^{-2} \text{s}^{-1}$  at 100%. The other emitters and detectors are built on the periphery of the unit. The data acquisition for the three signals, PF and MR in the light, and DF in the dark, is every 0.01 ms

in the digitalization range 1 (0.01–0.3 ms), every 0.1 ms in range 2 (0.3–3 ms), every 1 ms in range 3 (3–30 ms), and then decreases until range 7 (30–300 s), where the data acquisition is every 10 s.

During one measure, the measuring cycle includes light and dark periods in the ratio of 3:1. During the cycle, the PF is measured when the actinic light is on and DF is recorded when the light is off.

#### *Simultaneous Measurements of the Kinetics of Prompt Fluorescence, Delayed Fluorescence and Modulated 820 nm Reflection*

The typical polyphasic O-J-I-P curve of the increase in chlorophyll fluorescence is shown in Figure 2. The fluorescence rise up to the J-step provides information about single turnover events of the primary reactions of photochemistry, mainly QA reduction [13]. During the time interval from 2 to 200 ms, multiple charge separation occurs and the redox components of the electron transport chain become reduced [28]. The I-P phase is related to PhS I activity. For modulated reflection signals the first reliable MR measurement was at 0.3 ms. The MR decrease exhibits photoinduced oxidation of P700 and accumulation of  $\text{P700}^+$  and  $\text{PC}^+$  until about 20 ms, this accumulation is in the range of the J-I phase of PF. Subsequently, the MR increase exhibits re-reduction of both  $\text{P700}^+$  and  $\text{PC}^+$  by the intersystem electrons in the range of the I-P phase of PF [28].



**Figure 2:** Kinetics (induction curves) of prompt and delayed fluorescence (PF and DF, in different a.u.; left vertical axis) and modulated 820 nm reflection (MR; right vertical axis) induced by a 30 s pulse of strong red actinic light (627 nm peak,  $5000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) measured simultaneously with M-PEA and plotted on logarithmic time scale from 20  $\mu\text{s}$  to 30 s (JIP-time). The modulated reflection signals are expressed by the  $\text{MR}/\text{MR}_0$  ratio, where  $\text{MR}_0$  is the value at the onset of the actinic illumination (taken at 0.7 ms, the first reliable MR measurement). The DF induction curves (DF vs. JIP-time) were constructed from the kinetics of DF vs. delay-time recorded during the dark interruptions of the actinic light; each of them plots the DF intensity (a.u.) at a certain delay-time-point (indicated by the colour/symbol code) vs. the JIP-time at which the dark interval started. Hence, a vertical line cutting the DF induction curves at any JIP-time (like the three black

dashed lines in the plot) expresses (in one dimension) the kinetics of DF vs. delay-time in the dark interval that starts at the corresponding JIP-time. Characteristic points of the DF vs. JIP-time curves, i.e., the peak I<sub>1</sub> (at 7 ms), the shoulder I<sub>2</sub> (at 100 ms) and I<sub>3</sub> (taken at 1 s, in the plateau) are marked with open circles on the DF0.02ms (DF at 0.02 ms delay-time point) curve and labelled. Open circles were also used to mark the O, J, I and P steps of the PF kinetics.

The DF induction curves, shown in Figure 2, are averaged DF values collected within different DF delay-time intervals during the dark period after interruptions of the actinic light (the analytical time within each dark interval, during which DF is recorded, was noted as delay-time). Energy level diagram for the PhS II-states participating in DF generation has been presented by Goltsev et al.. In the DF induction curve, two phases can be observed: the fast one until 200 ms includes the I<sub>1</sub> and I<sub>2</sub> peaks, and the slow one until several minutes [6].

The DF curve measured at 10-30 ms delay-time consists of a fast rise to a peak I<sub>1</sub> (at 7 ms), a subsequent decrease through I<sub>2</sub> (at about 100 ms), and I<sub>3</sub>, sometimes found at the end of the fast phase. In slow-decaying components, concomitant with the disappearance of the first two maxima (I<sub>1</sub> and I<sub>2</sub>) in the induction curve, an appearance of the peak I<sub>3</sub> was observed, and this is mainly due to the slow millisecond components of DF. Goltsev et al. [6] suggested that the I<sub>1</sub> maximum is a result of the rise of

the transmembrane electrical gradient and of the accumulation of RCs with semi-reduced QB (Z<sup>+</sup>P680QAQB<sub>2</sub>), while I<sub>2</sub> was associated with the increase of Z<sup>+</sup>P680QA<sub>2</sub>QB<sub>2</sub> states during PQ pool reduction. Zaharieva et al. [23] reported that the I<sub>2</sub> maximum was probably related to the prolonged reopening of PhS II RCs by the electron transfer from the reduced QB to PQ before the reduction of the PQ pool. They suggest that the relative size of this maximum increases with the decrease of the size of the PhS II antenna and when the measuring temperature is increased.

In Figure 2, I<sub>1</sub> is between the J-step towards the I-step of the PF curve and in the oxidation phase of MR measurements and corresponds to the time of decrease of MR (7-10 ms). I<sub>2</sub> appeared in the range of the I-P phase of the PF and the re-reduction phase of MR measurements. I<sub>4</sub> appeared during the decline of the PF curve. The three latest DF induction curves (DF vs. JIP-time) the peaks I<sub>1</sub> and I<sub>2</sub> disappeared.

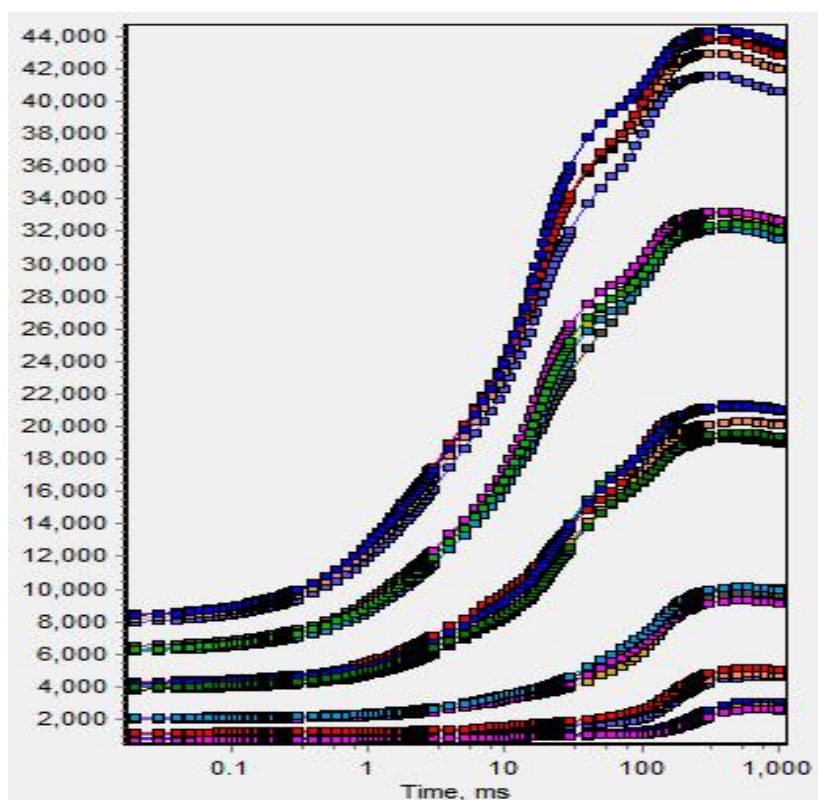


Figure 3: Visualization through the m-PEA-data-analyzer V.5.



In Figure 3 shows the direct view of the PF curves obtained from m-PEA during the measurements on the olive leaves (protocol: PF-1s 0300  $\mu\text{E DF}$  - single MR-100). The series is taken during the varies of light intensity 300, 500, 1000, 2000, 3000 and 4000  $\mu\text{E}$ .

A quantitative analysis of the O-J-I-P transient has been introduced [31] and further developed called as the 'JIP-test' after the basic steps of the transient, by which several selected phenomenological and biophysical- structural and functional-parameters quantifying the PhS II behavior are calculated. The JIP-test, has proven a very useful tool for the in vivo investigation of the adaptive behavior of the photosynthetic apparatus and, especially, of PhS II to a wide variety and combination of stressors, as it translates the shape changes of the OJ-I-P transient to quantitative changes of the selected parameters [29]. Hence, the JIP-test can provide an access to the 'vitality' of a photosynthetic sample [33].

The possibilities to obtain information on the condition of the photosynthetic apparatus (PhSA) from the shape of the induction curve are summarized in [30].

### 3. Results and Discussion

The purpose of this work was to study the reaction of the photosynthetic apparatus of olive plant, influenced by the intensity of actinic light. If the light intensity absorbed by the photosynthetic pigments is too high, all the absorbed energy can not be used in the process of photosynthesis. When the absorption rate of light quanta significantly exceeds the possibility of using their energy for photosynthesis, a condition called light stress occurs in plants. As a result of light stress occur functional disturbances in photosynthetic reactions (photoinhibition) and even damage to the photosynthetic apparatus can occur. Under conditions of low light intensity, most of the absorbed light energy can be used in the process of photosynthesis (due to the high efficiency of the photosynthetic apparatus), while at the same time only a small fraction of the FAR is used with high intensity [15]. Growing in bright light helps plants adapt to excess light energy and develop different acclimatization adaptation mechanisms that serves mainly to protect the photosynthetic apparatus.

We wanted to trace the plant cell's response to alter its features so as to ensure its optimal functioning under the new conditions (changed light intensities). For this, the leaves of olive plants were subjected to a change in light intensities and are recorded the induction curves of chlorophyll fluorescence from which were calculated the parameters of the JIP test, and  $\text{MR}_{820}$  and DF are recorded simultaneously.

In the present study is evaluate the effect of actinic light intensity ( $\approx 625 \text{ nm}$ ) on the PhSA in the olive leaves for induction transition from different time intervals. A dark adaptation was made in 1 hour. The intensities used are: 300, 500, 1000, 2000 and 4000  $\mu\text{mol h}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

In Figure 4/a are presented the induction transitions of PF, registered in the range of 0,01 ms to 1000 ms and Figure 4/b the induction curves (IC) of PF, recalculated as relative variable fluorescence ( $V_i$ ). The dependence of induction transition from light intensity is easily noticeable. With the his decrease, the induction curves lose its characteristic stepped shape, the values of the respective phases (J, I, P) decrease and the time to reach them increases (see  $F_P$  and the time at which occurs the  $t(F_P)$  in Figure 4/c and 4/d). It should be noted that the registered maximum fluorescence ( $F_P$ ) is equal to the maximum reached ( $F_M$ ) at 100% closed reaction centers (RC) only at an intensity  $1500 \mu\text{mol h}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

The light intensity strongly affects most parameters of the JIP test (Figure 4/c):

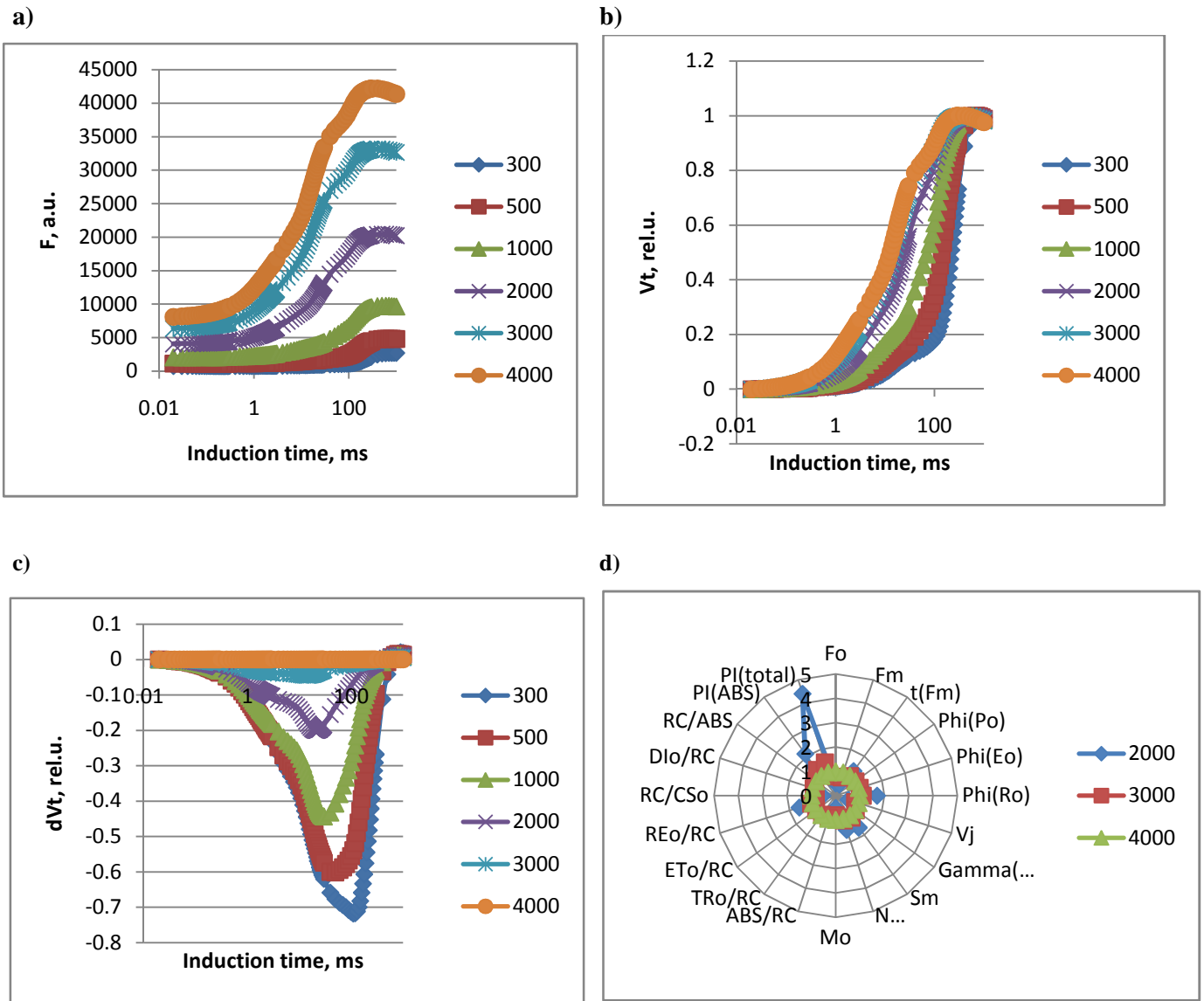
$\text{ABS/RC} = M_0 (1/V_J) (1/p_0)$  - the energy flux absorbed by one active reaction center (RC); reflects the ratio between the number of molecules of Chl a in antenna complexes emitting fluorescence, and in active reaction centers;

$\text{TR}_0/\text{RC} = M_0 (1/V_J)$  - the excitation energy flux captured by one active reaction center (RC) at the initial moment of illumination of the object adapted to darkness, that is, at  $t = 0$ ;

$\text{ET}_0/\text{RC} = M_0 (1/V_J)$  - the flux of electrons transported through one active reaction center (RC) at  $t = 0$ ;

$\text{RE}_0/\text{RC} = M_0 (1/V_J) (1 - V_i)$  - the flux of electrons transported through one active reaction center (RC) and reducing the outer acceptors on the acceptor side of the PhS I, at  $t = 0$ ;

$\text{DI}_0/\text{RC} = (\text{ABS/RC}) - (\text{TR}_0/\text{RC})$  - the total energy dissipated by one reaction center (RC) in the form of heat, fluorescence, or transfer to another photo system, at  $t = 0$ .



**Figure 4:** Effect of luminous intensity on PhSA in olive leaf. Dark-adapted for 1 h. olive leaves are illuminated with red actinic light with an intensity from 300 to 4000  $\mu\text{mol h}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Presented: a) IC of  $F_t$  a.u., Prompt Fluorescence (PF) at time  $t$  after onset of actinic illumination; b) IC of relative variable fluorescence,  $V_t = (F_t - F_o) / F_v$ , where  $F_t$  is the value at the respective time of registration,  $F_o$  - the initial and  $F_v$  - the variable fluorescence; c)  $V_t$ , rel.u.; d) PF parameters from the JIP-test relative to the values at 4000  $\mu\text{mol h}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

As the intensity increases, the specific energy fluxes (ABS/RC,  $TR_o/RC$ ,  $ET_o/RC$  and  $DI_o/RC$ ) as well as the RC density ( $RC/CS_o$ ) increase, while  $RE_o/RC$  and the probability, given chlorophyll molecule of PhS II to function as RC( ), decreases.

From the quantum yield of the electron transport from PQ to electron transport chain (ETCh) ( $\Phi_{R_o}$ ), end acceptors it is clear, that light-dependent reactions are most effective in the weakest illumination - 300  $\mu\text{mol h}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . This intensity falls within the ecological norms. During the day, the quality and amount of photosynthetic active radiation vary widely, and the plants try to find the balance

between the absorbed radiation energy and the photosynthetic apparatus. Intensities greater than 2000  $\mu\text{mol h}^{-1} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  are rarely observed in nature, but due to the fact that they quickly and effectively provide complete reduction of transporters in ETCh of PhSA, they are preferred in conducting fluorescence experiments with plants. The quantum yields of the primary photochemical reaction (PhChR) in the RC of PhS II ( $\Phi_{P_o}$ ) and the reduction of PQ by  $Q_A$  ( $\Phi_{E_o}$ ) are less influential.

The changes in MR 820 are shown in Figure 4/e. Analyzing the decline in the first part of the IC (up to 30 ms) it is noticed that the stronger the









