Temperture Effects on Olive Plants probed by Simultaneous Measurements of the Kinetics of Prompt Fluorescence, Delayed Fluorescence and Modulated 820 nm Reflection

Emilia CHACHI

Departament of Physics, "Ismail Qemali" University Vlora, Albania

Abstract-The reaction of the photosynthetic apparatus in higher plants to high - and low temperature could be analyzed, using simultaneous in vivo measurements of prompt fluorescence a, delayed fluorescence and modulated 820 nm reflection. Segments of olive plant leaves were placed on a metal surface at temperatures 10° and 5°C (low-temperature jump) or 30°, 35°, 40°, 45°, 50°, 52.5° and 55°C (highjump). temperature 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 photosystem 2, the photosystem 1 and the electron transport chain between the two photosystems; the concentration of the active reaction centers of photosystem 2; the electronic capacity of the electronic transport chain; as well total parameter that characterizes the the productivity in photosynthetic apparatus initial reactions. It is show that the low temperature temporary lower the photosynthetic efficiency of the electron transport, while the high temperature at above 40°C induced inactivation processes of the photosynthetic reactions. The sensitivity of the different sites of the electron transport chain to the heat decreased in the following order: (RC of photosystem 2) > (QA-PQ- pool) > (PQ.H2-PCphotosystem 1 - acceptors of photosystem 1).We suggest the simultaneous measurement of prompt and delayed chlorophyll fluorescence and 820 nm light reflection on leafs in vivo by Multi-Function Plant Efficiency Analyser, that allows accurate estimation of the physiological state of the plants and provide useful information on assessing the degree of plant tolerance to different environmental conditions.

Keywords—temperture effects, chlorophyll fluorescence, prompt chlorophyll fluorescence, delayed chlorophyll fluorescence, modulated light reflection 820 nm.

1. Introduction

Plants, as an open thermodynamic system, exist in the conditions of permanently changing environment. The power of external influence determines to a great extent the final effect on the plant organism. Strong effects of biotic and abiotic environmental factors are capable of causing significant structural and physiological changes or damage to plants. At the same time, low intensity factors modify the variable functional characteristics of the plant cell, ensuring maximum efficiency of the processes running in the organisms (Strasser et al., 2000). Photosynthesis is the most important energetic process in plants. Luminous photosynthetic reactions are very sensitive to changes in external conditions, so they can be used as a model for studying the reaction of plants. Strasser et al. (Tsimilli-Michael and Strasser, 2008; Strasser et al., 2010; Goltsev et al.,2010) have developed an approach used to characterization the function state of native plant systems (whole plants in vivo and in situ), based on studying of photoinduced changes in chlorophyll a fluorescence in the plant tissues.

The derivation of links between the energetic behavior of the photosynthetic apparatus and the fluorescence signals in our approach, is based on the 'Theory of Energy Fluxes in Biomembranes' [23, 24]. 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 photosystem 2 (PhS) units, as well as from PhS 2 to PhS 1.

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 [26]) 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 [10] reflect three different reduction processes of the electron transport chain [25]. 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 2, and PhS 1 contributes very little to the DF emission. PF depends on the redox state of the PhS 2 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 QA⁻ and the oxidized donor (P680⁺) of PhS 2 that are formed after light-induced charge separation [26]. 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 backward electron transport reactions in the RC of PhS 2. 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.

Energy cascade: energy fluxes, bifurcations and energy conservation efficiencies	Definition of events and corresponding energy fluxes	Definition of quantum yields & efficiencies	Correlation with fluorescence signals		
ABS	<u>Abs</u> orption by PSII antenna pigments	$\phi_{Pt} = \Delta F_t / F_M = \frac{TR_t}{ABS} = 1$ actual quantum yield at any time t	$-\frac{\mathbf{F}_{t}}{\mathbf{F}_{M}} = \boldsymbol{\varphi}_{Pt} = \boldsymbol{\varphi}_{Po} (1 - \mathbf{V}_{t})$ (according to Paillotin [21])		
$\begin{array}{c} C \\ ABS-TR \\ (1-\phi_P) \end{array} TR \\ \phi_P \\ \hline \end{array}$	<u>Tr</u> apping (primary photochemistry): reducing Pheo and Q _A	$\phi_{P_0} = \phi_{P_0} \qquad \qquad = \frac{TR_0}{ABS} = 1$	$-\frac{F_0}{F_M} = \phi_{P_0} = \phi_{P_0} (1 - V_0)$		
$\begin{array}{c} \mathbf{TR} \cdot \mathbf{ET} \\ \mathbf{TR} \cdot \mathbf{ET} \\ (1 - \psi_E) \end{array} \qquad $	<u>E</u> lectron <u>T</u> ransport after Q_A^- to intersystem electron acceptors (and other pathways)	$\varphi_{E_0} = \varphi_{P_0} \cdot \psi_{E_0} \qquad = \frac{ET_0}{ABS} = 1$	$l - \frac{F_J}{F_M} = \phi_{Pj} = \phi_{Po} \left(1 - V_J \right)$		
$\begin{array}{c c} \hline Q_B T Q C V T C (X) \\ \hline ET - RE \\ (1 - \delta_R) \\ \hline Ed NADP \\ \hline \end{array} \\ \hline \end{array} \\ \begin{array}{c c} \varphi_R \\ \varphi_R \\ \varphi_R \\ \hline \end{array} \\ \hline \end{array}$	<u>R</u> eduction of <u>E</u> nd acceptors (at PSI electron acceptor side)	$\phi_{Ro} = \phi_{Po} \cdot \psi_{Eo} \cdot \delta_{Ro} = \frac{RE_0}{ABS} = 1$	$1 - \frac{F_{I}}{F_{M}} = \phi_{Pi} = \phi_{Po} (1 - V_{I})$		
$\mathbf{PI}_{ABS} = \frac{\gamma_{RC}}{1 - \gamma_{RC}} \cdot \frac{\phi_{Po}}{1 - \phi_{Po}} \cdot \frac{\psi_{Eo}}{1 - \psi_{Eo}} = \mathbf{PI}_{ABS} \cdot \frac{\delta_{Ro}}{1 - \delta_{Ro}}$	$= \frac{\mathbf{RC}}{\mathbf{ABS}} \cdot \frac{\mathbf{TR}_{0}}{\mathbf{ABS} - \mathbf{TR}_{0}} \cdot \frac{\mathbf{ET}_{0}}{\mathbf{TR}_{0} - \mathbf{E}'}$ $= \mathbf{PI}_{ABS} \cdot \frac{\mathbf{RE}_{0}}{\mathbf{ET}_{0} - \mathbf{RE}_{0}}$	$\overline{T_0} = \frac{RE_0}{ET_0} = \frac{ET_0}{TR_0} = E$	$\frac{(\mathbf{F}_{\rm M} - \mathbf{F}_{\rm I})}{(\mathbf{F}_{\rm M} - \mathbf{F}_{\rm J})} = \frac{(1 - \mathbf{V}_{\rm I})}{(1 - \mathbf{V}_{\rm J})}$ $\frac{(\mathbf{F}_{\rm M} - \mathbf{F}_{\rm J})}{(\mathbf{F}_{\rm M} - \mathbf{F}_{\rm 0})} = (1 - \mathbf{V}_{\rm J})$		
The specific energy fluxes (fluxes yields (fluxes/ABS) and ABS/RC	(RC) are the products of quantum c, experimentally determined as:	The relative variable fluorescence V_t is defined as:	The total electron carriers (EC_0) per RC is defined as:		
$\frac{ABS}{RC} = \frac{TR_0/RC}{TR_0/ABS} \text{ where } \frac{TR_0}{R}$	$\frac{R_0}{C} = \frac{(\Delta F / \Delta t)_0}{F_J - F_0} = \frac{4(F_{300\mu s} - F_0)}{F_J - F_0}$	$\mathbf{V}_{t} = \frac{\mathbf{F}_{t} - \mathbf{F}_{0}}{\mathbf{F}_{M} - \mathbf{F}_{0}}$	$\frac{\mathbf{EC}_{0}}{\mathbf{RC}} = \frac{\mathbf{Area}}{\mathbf{F}_{\mathrm{M}} - \mathbf{F}_{0}}$		

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

Temperature is one of the most important environmental factors determining the functioning of plants. The influence of temperatures (predominantly elevated) on photosynthetic apparatus (PhSA) functioning is expressed by changes in the ability of oxygen separation (see Nash et al., 1985; Enami et al., 1994) in primary electron transport in the thilacoid membranes (TM) (Frolec et al ., 2008; Koushil et al., 2004) or in the assimilation of carbon dioxide (Crafts-Brandner and Salvucci, 2002; Lazberg et al., 2005).

In the present work we traced the complex reaction of the PhSA in the leaves of olive plants to low (10, 5°C) or high (30, 35, 40, 45, 50, 52.5, 55°C) temperature, using three signals - PF, DF and modulated reflection MR 820 nm. We have shown

that simultaneous measurement of PF, DF and MR is an efficient instrument to characterize the influence of temperatures 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 experiments were used Olea Europea L. Sativa, "Kalinjot" and "Ulliri i holle i Himares" seedlings, autochthonous cultivars, native plants grown in nature, which 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°C, day/night mode 12: 12h and luminescent lighting with intensity 250 µmol hv. m⁻²s⁻¹, close to natural ecological radiation.

Influence of temperature on photosynthetic organisms

Plants, growing in the Mediterrameon region, are relatively wide fluctuations subjected to of temperature and experience stress at both high and low temperatures (McKersie and Leshem 1994). Under such conditions, the saturation of the hydrocarbon chain in fatty acids of membrane lipids and the resistance of membranes can influence the efficiency of photosynthetic electron transport. The chlorophyll a fluorescence kinetics analysis is an instrument often used to study the effects of various environmental stresses on photosynthesis. (Kalaji et al, 2004 ;. Kalaji et al, 2012; ..Goltsev et al, 2012; Yordanov et al, 2012). Low temperature disturbs the function of the photosynthetic apparatus (Öquist et al.1987). An inhibition of sucrose synthesis is observed, resulting in reduce recycling of phosphate and phosphorylation (Labate and Leegood 1988) and retardation of photosynthetic electic transport (Savitch et al., 1997). Low temperatures increase the probability of photoinhibition (Goodde and Bornman 2004). During the stress caused by frost, changes in chlorophyll a fluorescence parameters such as PIABS, ET/CS, AM (zone) and RC / CS (Rapacz, 2007) are observed. The adapt during growth at low temperature is related to the regulation of maximum photosynthetic capacity (Adams et al., 2001). This adjustment includes itself an increase in the distribution of thermal energy (heat) (Demmig-Adams et al., 1996) and the content of the hydrophobic proteins PsbS, which are included in the distribution process of thermal energy (Li et al., 2000). This leads to declining generation of reactive oxygen forms, such as hydrogen peroxide and singlet oxygen, during plant growth at low temperatures (Morgan-Kiss et al., 2006). The increased tolerance of plants to low temperatures is provided by an adaptive mechanism that supports the fluidity of the membranes in physiological boundaries (Morgan-Kiss et al., 2006).

The reduction of fluorescence under stress from high temperatures is caused by damage of thylakoid membranes (TM) and reduction of PhS 2 activity (Harding et al., 1990; Ilík et al, 2003; Weng and Lai, 2005). The increase in time to achieve maximum fluorescence (T_{FM}) at the high temperature conditions indicates that there is a blockage of energy transfer from reaction centers to plastoquinonyl (Reigosa and Weiss, 2001).

The high temperature significantly increases the permeability of the membranes, damages PhS 2 subunits and reduces oxygen release activity as a result partial damage to Mn-complex of PhS 2. The latter leads to a limitation of the electronic transport in

the donor side of PhS 2, the indicator of which is the appearance of the "K - peak" in the IC of PF.

The damage of TM and the increase in permeability are not caused only by peroxidation of their lipids, but rather result from: membrane protein conformational changes, ion channels opening, changes in lipid interactions, redistribution of lipids in TM (Santarius 1980; Havaux et al., 1996) as well as formation of monolayer segments from the lipid phase of the membrane (Gounaris et al., 1984, Kóta et al., 2002). The occurrence of K-peak in induction curve (IC) of PF in plants, growing at high temperature indicates for partial damage to Oxygen Evolving Complex (OEC) (Strasser et al., 2005). The reason for its occurrence is probably an imbalance between the transfer rate of electrons from tyrosine YZ to the oxidized chlorophyll P680⁺ in the reaction center (RC) of PhS 2 and the rate of reoxidation of QA and QB. By reducing the speed of electron transport reactions in the donor side of PhS 2, reseparation of the charges may lead to the accumulation of P680⁺ or to their rapid recombination (Strasser 1997).

The high values of ABS/CS (F_0), which are observed at high temperatures, may be related to the dissociation of light-gathering complex (LHC) to PhS 2 (Schreiber and Armond, 1978, Yamane et al, 1997).

The low values of the F_M parameter in low or high temperature show that the size of electron acceptors restoration pool in PhS 2 (mostly QA) is reduced. The same effect of decreasing the F_M value can be observed in the case of blocking the transport of electrons from the reaction centers (RC) to the pool plastohinon (Schreiber et al, 1989, Hansatech, 2000).

High-temperature stress reduces PhS 2 activity, which can be seen from phenomenological parameters (absorbed flows and energy captured by RC energy as well as electron transport flows) normalized for unit of illuminated leaf surface, which undergoes important changes.

The results of various studies show that the PI(ABS) parameter is the most sensitive indicator of the influence of various stress factors, including temperature (Goltzev et al., 2014).

Working with mPEA

The simultaneous registration of the photoinduced signals of prompt and delayed chlorophyll a fluorescence and the swallowing changes at 820 nm was realized with the Multifunctional Plant Efficiency Analyzer (m-PEA), developed and manufactured by Hansatech Instruments Ltd. (King's Lynn, Norfolk, UK) (Figure 2).

Changes in the characteristics of the induction curves of chlorophyll fluorescence, caused by temperature changes of the object, were investigated using an experimental system, including a m-PEA fluorimeter, a thermostatic unit, allowing to maintain on working table a temperature in the range from -10° C to $+70^{\circ}$ C.



Figure 2: Experimental setup for investigation of "temperature jump" effects on leaves.

After a darkening for 1h the olive plant, a torn leaf is placed on the holder, providing contact with the surface of the metal plate of the thermostatic block, with the underside of the leaf on the contact plate. The temperature of the plate adjust to an accuracy of \pm -0,1°C. The working head of m-PEA, mounted on a movable support, is placed on the unit. The results obtained are analyzed with the software program of the fluorimeter m-PEA.

Measurement Protocols

30s after being placed on the tempered plate, the subject under test is illuminated by a series 40 pulses of light an intensity of 4000 µmol hv. m⁻²s⁻¹, alternated with a dark interval between the individual pulses. Transitions caused by jump in high (from 24°C to 30, 35, 40, 45, 50, 52.5, 55°C) and low (from 24°C to 10, 5°C) temperature are analyzed. The induction kinetics of PF and DF are recorded. The kinetics of scattering of the modulated infrared light with 820 nm (MR820) is obtained in red and infrared light, the intensity of which is the maximum (100%) that the m-PEA device can provide. The 100% intensity of infrared light corresponds to approximately 700 μ mol hv.m⁻².s⁻¹ red light, i.e. infrared light is much weaker. For each PF induction curve, the JIP-test parameters are calculated, the quantum yields of electron transport reactions in the reaction center (RC) of PhS 2 (ϕ PO), the acceptor side of PhS 2 (qEO) and between the polyacidone pool and the PhS 1 acceptors (ϕ RO).

3.Results and Discussion

The purpose of the study was to analyze the PhSA reaction in high plants during the application of relatively poor stress, which does not cause significant structural modifications, and irreversible damage to cellular functions. We want to follow the ability of the plant cell to change its characteristics to ensure optimal operation under the new conditions (by temperature change). Evaluation of the effect of high and low temperatures on PhSA on olive leaves was made according to:

- the induction curves of prompt and delayed fluorescence, ;

- the kinetics of modulated 820 nm reflection;

- the parameters from the JIP-test of prompt fluorescence;

- the parameters of modulation distribution at 820 nm.

In Figure 3a shows the IC of PF (absolute values), and in b - Vt, variable fluorescence (relative values). With the temperature rises, the F_M decreases (Figure 3a, c, Tabele 1). F_M increases by about 20% at jump in low temperature from 24 to 5°C and decreases by about 30% at jump in high temperature from 30 to 55° C. The reason for this may be denaturation of the pigment-protein complexes (decrease of RC/CS₀), or increase of nonextinguishing, photochemical due the to accumulation of P680⁺. The latter phenomenon is easily illustrated by increasing the K-peak at elevated temperature (Figure 3a,e). This peak is clearly visible in the IC of PF at 50°C, and this way the first time registered by Bruno Strasser (Strasser 1997), which explains it with damages in the structure and function of oxygen evolving complex (OEC). As the non-photochemical temperature rises, the extinguishing after P (Figure 3d) is also increased. The negative K-peak at low temperatures is associated with a slow ET at the acceptor side of PhS 2, for the reason of delayed exchange between the QB and the PQ- pool, due to the reduced diffusion of PQ molecules in TM. The latter is confirmed by the high values of J and T_{Fm} at 5 and 10°C (Tabele 1, Figure 3c).

noted. It should be that the primary photochemical reaction (PPhR) quantum efficiency of P680 (ϕ_{Po}) is almost unchanged from 5 to 45°C, indicating, that in this temperature range, the protein complexes of the integral scheme RCs of PhS 2 are stable. According to literature, the most sensitive is the electronic transport between the two systems, judging by φ_{E_0} and φ_{R_0} . In our measurements φ_{E_0} for the same temperature range from 5 to 45°C almost does not change, while φ_{Ro} does not change from 24 to 40°C and at intervals from 5 to 10°C and from 45 to 55°C the values are raised, which shows similar reacts in electronic transport between two systems in two extreme temperature ranges.

Another characteristic peculiarity at severely damaging temperatures (55°C) is the appearance of a high initial value (F_0) (Figure 3a, c, Tabele 1) due to the dissociation of the antenna complexes, light harvesting complex (LHC) from PhS 2 (Yamane et al., 1997). By comparing the changes in the L-peak (Figure 3f), the disintegration trend of the antennas is evident, even at high temperatures, slightly damaging. At the reduced temperatures, the reverse process is observed - the grouping increases due to

the fluidity decrease of TM. The observations so far show a high temperature sensitivity of PhS 2. In our measurements the clustering process is observed in the temperature ranges from 5 to 10°C and from 45 to 52.5°C.

IC of MR 820 provide information on the temperature dependence of the redox reactions in PhS1. From the signal can be calculated the characteristic rates of oxidation-reduction (Vox. Vred). The amplitude of the RC oxidation of PhS 1, as well as the time to reach it. decreases as temperature increases (Figure 4 a). Consistent with this, is the decline of P700 (Vox) oxidation rate and simultaneously is rising the P700⁺ (Vred) reduction rate (Figure 4b, Tabele 2). It should be noted that the two speeds differ with one order among them. Their dependence on temperature is different: from 5 to 30°C Vox is decreased very slowly, Vred grows about 10 times faster compared to Vox; from 30 to 50°C Vred size decreases by 95% and Vox continues to decrease about 4 times slower. Since Vox reflects only the primary photochemical reaction (PPhR) in the RC of PhS 1, it follows that the latter is affected less from the temperature in contrast to the other components of electron transport chain (ETCh), especially PhS 2. In fact, inhibition of PhS 1 begins barely after 30°C, and low temperatures do not adversely affect it. Raising of Vred is related to "liquefving" the membrane and facilitating the diffusion of intermediate electronic carriers. After 45° C the strong damage to PhS 2 leads to a reduction in Vred.

The differences in the course of Vred in various illumination are explained by the fact that at high luminous intensity the withdrawal of the electrons from the PQ - pool to PhS 1 is limiting, and at low intensities limitation of the reduction of the pool of

PhS 2. The latter is highly sensitive to high temperatures.

We did not observed any change in shape and intensity of the PF and DF curves when the temperature was increased up to 40° C. At temperature 45 and 50° C we observed a decrease in the amplitude of the PF curve and a change in its shape. At 55° C temperature the amplitude of the PF curve is reduced 2.4 times compared to the 24° C control and the characteristic shape of the IC breaks.

We observed that the DF curves are more affected than PF curves at temperatures of 25, 30°C (Figure 5a-d). This may mean that DF curves are more thermosensitive than PF curves. Indeed, at these temperatures, we observed a decrease in the amplitude of the DF curve and change of I_1 , I_2 and I_3 peaks obtained from DF10-30 ms, while minor significant changes in PF curves were monitored. At relatively high temperatures, the DF signal falls below the observed control level. Even higher temperatures (45, 50°C) cause a decrease in the peaks yields at I_1 (7 ms) and I_2 (100 ms), disappearance of DF decay from I_1 to I_2 and the disappearance of the I_1 peak at 50° C. The disappearance of DF decay between the I_1 and I_2 peaks occur in parallel to the disappearance of the J-I phase from the PF curve. The relative I2 in the fast DF phase increased and the relative DF induction increased in the slow phase. The increase of relative DF in the slow phase might be related to the activation of the Calvin-Benson cycle. These observations have been reported by Zaharieva et al [30]. The maximum I₄ occurs in parallel with a decrease of the PF intensity. We noted here that I₄ was less affected by heat. At low temperatures from 10 to 5° C are seen changes in the characteristic form of IC of DF (Figure 5a, c), increase of amplitude (amplitude at 5° C is twice the amplitude at 30° C).





Figure 3: The effect of temperature over chlorophyll a fluorescence on olive seedlings. Olive leaves adopted for 1h in the dark are placed on the thermostat at a regulated temperature of $5-55^{\circ}$ C and after 1 min incubation, induction curves of PF are recorded at 4000 µmol hv.m⁻².s⁻¹ excitation light intensity. Appear:

a) IC of PF, absolute value;

b) IC of relative variable fluorescence (Vt):

c) JIP-Test parameters, referred to 24°C;

d) Changes in the IC form of Vt for the entire measurement period, as the reference is used the taken signal at 24° C – Vt (24);

e) K-peak: changes in the IC of Vt to the O-J interval;

f) L- peak: changes in the IC of Vt to the O-K interval.

Table '	1
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	Fe	Fm	t(Fm)	φ(Po)	φ(<u>E</u> ρ)	φ(Ro	<u>Vi</u>	χą(RC)	Sm	N(Str)	Mo	ABS/RC	TRo/RC	ETo/RC	RE0/RC	RC/CSo	DIo/RC	RC/ABS	PI(ABS)	PI(totall
÷	1.125	1 207	1.462	1.000	0.004	1 000	1.000		1.051	1 000	1 1 1 2	1 010	1.025	1.002	1.050	1.166	0.001	0.001	0.002	1 402
2	1.175	1.207	1.465	1.006	0.984	1.552	1.088	0.99	1.851	1.899	1.115	1.019	1.025	1.003	1.559	1.155	0.991	0.981	0.905	1.497
10	1.064	1.089	1.439	1.005	1.012	1.416	0.973	1.001	1.791	1.803	0.976	1	1.005	1.012	1.418	1.066	0.977	1.002	1.057	1.866
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24K	0.954	0.919	0.707	0.992	1.006	0.929	0.944	0.934	0.831	0.937	1.063	1.136	1.127	1.142	1.055	0.842	1.179	0.883	0.9	0.792
30	0.979	0.966	0.829	0.997	1.001	1.054	0.985	0.953	0.948	1.027	1.112	1.102	1.098	1.095	1.14	0.882	1.118	0.919	0.989	1.103
35	0.909	0.892	0.732	0.996	1.027	1.111	0.871	0.96	0.913	0.979	0.937	1.079	1.075	1.108	1.198	0.841	1.101	0.927	1.07	1.246
40	0.981	0.948	0.585	0.993	1.017	1.16	0.901	0.94	0.8	0.891	1.003	1.122	1.114	1.141	1.301	0.876	1.16	0.892	0.967	1.194
45	0.944	0.869	0.585	0.982	0.983	1.317	0.993	0.928	0.763	0.858	1.116	1.146	1.125	1.127	1.509	0.825	1.245	0.872	0.791	1.286
50	1.094	0.817	0.383	0.928	0.807	1.331	1.531	0.863	0.652	0.789	1.845	1.303	1.207	1.051	1.741	0.844	1.756	0.771	0.305	0.76
52.5	1.01	0.605	0.456	0.851	0.729	1.484	1.58	0.719	0.84	1.233	2.363	1.743	1.483	1.268	2.577	0.575	2.971	0.576	0.161	0.822
55	2.044	0.699	0.395	0.594	0.459	1.413	1.931	0.382	1.385	3.42	4.705	4.159	2.439	1.885	5.859	0.5	12.3	0.248	0.021	-0.058



Figure 4: Temperature effect over the MR820 on olive leaves. The experimental conditions are the same as in Figure 3. Appear:

a) IC of MR820 to 4000 μ mol hv.m⁻².s⁻¹ red light; b) Rate of oxidation of P700 (Vox) and of reduction of P700 ⁺ (Vred), obtained from lines, approximating the MR820 signal from point a) to defined time interval.

Tabele 2

	5	10	24	24 K	30	35	40	45	50	52.5	55
Rate ox	2.82289	2.64386	2.57422	2.53756	2.51393	2.30475	2.67084	2.38838	2.09454	1.61827	1.83319
Rate red	0.01033	0.03996	0.11422	0.12358	0.13323	0.11799	0.08208	0.03268	0.00697	0.00697	0.00697





Figure 5: DF induction curves recorded at interval 10-100 µs after the start of exciting light, depending on temperature. a) DF absolute value, microsecond; b) DF relative variable, microsecond; c) DF absolute value, submillisecond; d) DF variable relative, submillisecond.



Figure 6: Visualization by m-PEA-data analyzer V.5.5 of DF kinetics at 0-099ms interval, result of averaging and processing of which is shown in Figure 5.

4.Conclusions

The photosynthetic apparatus in high plants responds sensitively to changes in environmental conditions. The connection of the plants with the temperature conditions determines the physiological state of their photosynthetic apparatus. Temperatures in the range of 0 to 50° C are common for vegetation of plant cultures in Albania and olive

plants are able to withstand short-term impacts. At the same time, immediate change in temperature after a continuous stay at 24° C to higher (30-50° C) or lower (10-5° C) causes complicated reaction in PhSA of plants, with the objective of adopting plant cells in the new conditions of functioning. By applying the metode of simultaneous measurement of chlorophyll a fluorescence and modulated reflection at 820 nm, we can follow PhSA's state at different moments when observe characterizing we parameters, among which the quantum effectiveness of the electrons flux in PhS 2, PhS 1 and in the transport chain between electrons the two photosystems; the concentration of active reaction centers at PhS 2, the electronic capacity in the electron transport chain; the total parameter, that characterizes productivity in initial reactions in PhSA.

Through the parameters of the JIP test we analyzed the stressful reaction of PhSA in the immediate change of temperature in leaves of olive saplings and studied the dynamics of crossings, induced by low and high temperature jump. In low temperature influence, the efficiency of electrons transport decreases fast in all the analyzed segments, after which take place processes, which compensate this decrease. In instantaneous heating up to 40° C, increased electron transport efficiency is observed, and at higher temperatures develop processes of inactivated of photosynthetic reactions. The analysis of the infrared light distribution signal (820 nm) shows the difference between PhSA reactions to high and low temperature. At the high temperatures, inactivation of the connection between the two photosystems occurs, where PhS 1 remains functional even at 50°C. Low temperature stress (24° C - 5°C) causes interim inactivation in the electronic transport between the systems.

The phenomenological PI (ABS) parameter, the most sensitive indicator of the influence of various stress factors, including the temperature, is stable in the range from 10 to 40° C, slightly decreases to 5° C, while from 45 to 55° C it is decreases by about 40 times.

The temperature is the basic ecological factor for plants. Its daily and annual amplitude is noticeably high on Earth. For the olive plants temperatures higher than 45 °C cause significant damages to the P680 (qPo) and weaker on the P700 (Vox), so PhS 2 is more thermolable than PhS 1. The low temperatures (5, 10)°C) reduce the nonphotochemical quenching. while the high temperatures magnify it. High sensitivity from temperatures indicates the electron transport (phase level J, φ_{Eo} and φ_{Ro}), possibly due to increased carriers energy, important for the transfer of electrons, as well as the presence of mobile carriers (PQ and PC), diffusion of which increases with temperature increase.

At high temperatures (50° C) , the real positive Kpeak is observed. In addition to OEC damages, at 55° C antenna complexes (LHCs) dissociate from RCs, judging by the rise of ABS / RC. The positive Lpeak show a decrease in the grouping between them since at non-destructive temperature. At low temperaments in the PhS 2 limited is ET between Q_A and Q_B, which is judged by negative K-peak, while the grouping increases - negative L-peak. The reduction of the P700⁺, due to both ETC and stromal donors, is accelerated by temperature. The same influence of the temperature effect on both reactions indicates, that the two reactions are determined by related enzymes.

The Influence of temperature jump, in low and high, overhead on native plants causes complex PhSA reaction with direction to protection from damage as well as and functional adaptation.

The simultaneous measurement of prompt and delayed fluorescence kinetics and modulated reflection in 820 nm is an information method for assessing of the reaction dynamics and the state of the plant. By applying the method, we follow the state of PhSA at different moments and through the JIP test parameters, we analyze the reaction of the photosynthetic apparatus during a change of ambient temperature in the olive plants.

5.References

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