Arch. Biol. Sci., Belgrade, 60 (4), 567-572, 2008 DOI:10.2298/ABS0804567N

EFFECTS OF TEMPERATURE AND LIGHT INDUCTION OF CHL A FLUORESCENCE IN SITU: AN ECOPHYSIOLOGICAL VIEW

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Abstract — The effects of PAR and temperature on induction of Chl a fluorescence were observed on leaves of 20 plant species in their natural environments. Light affects the acceptor side of PS2. Temperature regulation of PS2 activity proceeds by affecting the RC and acceptor side of PS2. The impact of higher temperature can be attributed to greater fluidity of the thylakoid membranes. Photosynthetically active radiation and temperature under the given conditions are in highly significant positive correlation, so it is not clear whether this impact is due to individual or complementary mechanisms of PAR and/or temperature affecting photo-synthetic processes in the thylakoid membranes. Also, it is not clear whether species specificity has any significance in the plant photosynthetic response to changes of PAR and temperature. Changes of PAR and temperature during induction of Chl a fluorescence do not affect Pindex, as a parameter of total photosynthesis.

Key words: Chl a fluorescence, PAR, temperature, RC and acceptor side of PS2, thylakoid lipids

UDC 581.132:574:57.08

INTRODUCTION

As a process of energy conversion, photosynthesis depends on the intensity (PAR) and quality (R/FR acclimation) of light. Photosynthetically active radiation or PAR is probably the most important variable environ-mental factor affecting plants (Björkman, 1981). This factor varies over seasons, days, and regions.

Plants adapt to the prevailing light system: there are sun-requiring plants (heliophytes) and shade-requiring plants (sciophytes) (Björkman, 1981). Generally, sun-requiring plants have greater intensities of photosynthesis in light with higher PAR than those observed in shade-requiring plants under these conditions (Björkman, 1981). Besides light, temperature is another important ecophysiological factor that affects photosynthesis (Berry and Björkman, 1980). Temperature affects photosynthesis through a series of enzymic reactions according to Michaelis-Menten kinetics (Edwards and Walker, 1983), as in a case of photosynthetic reactions in the stroma (dark phase of photo-synthesis). Moreover, temperature affects photosynthetic processes in the chloroplast membrane (light phase of photosynthesis), which mainly depends on the state of thylakoid lipids (Lyons et al., 1979; Nishida and Murata, 1996). Photosynthetic reactions within the membrane are related to photosynthetic processes in the stroma (Edwards and Walker, 1983; Geiger and Servaites, 1994). Total photosynthesis can therefore be estimated by observation of the light phase. Measuring Chl a fluorescence is a common technique for monitoring membrane photosynthetic reactions (Krause and Weis, 1991).This method is used to estimate the state of membrane reactions, as well as the level of total photosynthesis in various ecophysiological circumstances (Björkman and Demmig, 1987; Genty et al., 1989; Burke, 1990; Demmig-Adams and Adams, 1992; Oberhuber and Edwards, 1993). Ecophysiological studies on

effects of light and temperature on photosynthesis *in situ* are mostly done by so-called PAM fluorometry (Maxwell and Johnson, 2000). The application of non-modulated fluorometry is much less present in ecophysiological studies.

MATERIAL AND METHODS

Chl a fluorescence measurements, other measurements and statistical processing of obtained results

Induction of Chl a fluorescence induction was monitored with a Handy-PEA portable fluorometer (Hansatech, UK), which operates on the principle of non-modulated fluorescence.

This device has a software for calculation, numerical presentation, and memorization of Chl a fluorescence parameters. Parameters F_0, Fm, Fv/Fm and Fv/F_0 were observed as defined by Krause and Weis (1991), Strasser et al. (1995), and Maxwell and Johnson (2000). The Pindex parameter (photosynthesis relative vitality index) and parameters Tfm (msec; time taken to reach Fm, an indicator of Q_A reduction rate of the PS_2 acceptor, i.e., the rate of PS_2 electron transport) and A (area bmS; the area above the fluorescence induction curve between F_0 and Fm, measurement of size of the plastiquinone pool in PS_2), defined in accordance with Strasser et al. (1995), were also monitored. Photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}) was measured with a Li-1000 light meter (Li-Cor, USA), while temperatures were measured with a BIG DIGIT Dual Thermo thermometer (measuring range from -50 ºC to +70 ºC). As light and temperature represent the energy (light and thermal) to which plants are exposed, the obtained results were, prior to statistical processing, transformed by the method of Arrhenius as modified by Marković et al. (1996). This transformation of results was done by logarithmization of parameters not presented as ratios (F_0, Fm, Tfm, and A), while PAR and the absolute temperature (T; ºK) were given as reciprocal values (1/A, 1/T). Parameters defined as ratios (Fv/Fm, Fv/F_0, and Pindex) were not transformed. Statistical processing of results (determinations of means, correlation, and regression analyses of relations of fluorescence parameters vs. 1/PAR or 1/T) was performed in the Excel program package (Microsoft, USA).

Plant material and measuring conditions

In July of 2006, Chl a fluorescence was measured on healthy leaves of 20 cultivated and native plant species of various life forms *in situ* in Kraljevo [five species: sour cherry, thuja, fig, walnut, and rose; PAR≅1000 μmol m^{-2} s^{-1}; T≅35ºC; ≅200 m; 150 km south of Belgrade] and Rudno [15 species: common cocklebur and dog rose (PAR≅40 μmol m^{-2} s^{-1}); plum, apple, walnut, and pear (PAR≅65 μmol

Table 1. Correlation of parameters of induction of Chl a fluorescence and reciprocal values of absolute temperature (1/T; °K) and photosynthetically active radiation (1/PAR; μmol m-2 s-1) measured during July of 2006 in Kraljevo and Rudno on leaves of 20 plant species (*p<0.05, **p<0.01)..

Character	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) ln F_0								
(2) ln Fm	0.663**							
(3) Fv/Fm	-0.275	0.535*						
(4) Fv/F_0	-0.312	0.499*	0.985**					
(5) Pindex	-0.810**	-0.345	0.474*	0.489*				
(6) lnTfm; mS	0.054	0.116	0.103	0.041	0.076			
(7) ln A; bmS	0.125	0.443	0.443	0.384	0.177	0.802**		
(8) 1/PAR;μmol m^{-2} s^{-1}	-0.031	0.239	0.337	0.323	0.186	0.649**	0.588**	
(9) 1/T; ^{0}K	-0.180	0.394	0.698**	0.709**	0.274	0.490*	0.614**	0.769**

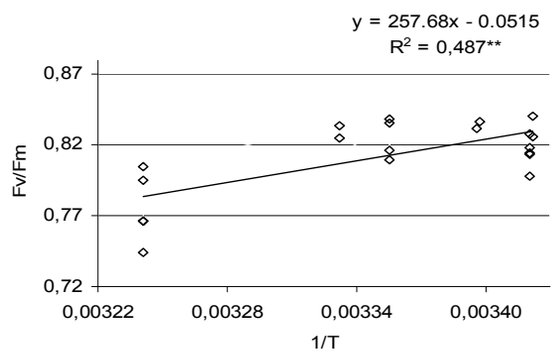

Fig. 1. Regression of Fv/Fm parameter of induction of Chl a fluorescence and reciprocical values of absolutne temperature (1/T; K). **p<0.01.

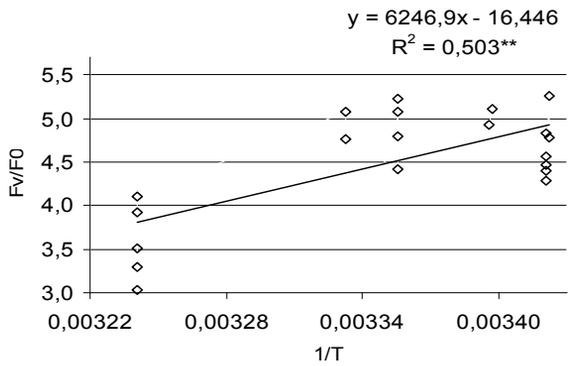

Fig. 2. Regression of Fv/F0 parameter of induction of Chl a fluorescence and reciprocical values of absolutne temperature (1/T; K). **p<0.01.

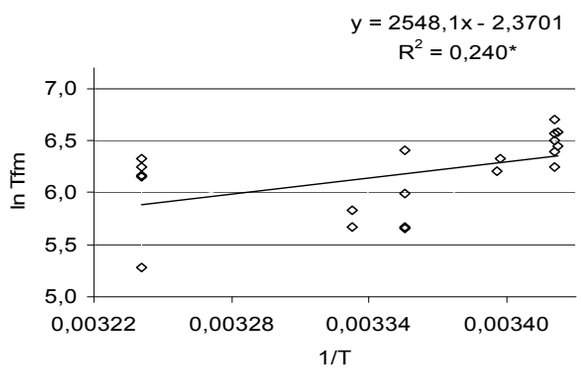

Fig. 3. Regression of ln Tfm (mS) parameter of Chl a fluorescence eter of Chl a fluorescence and reciprocical values of absolutne temperature (1/T; K). **p<0.01.

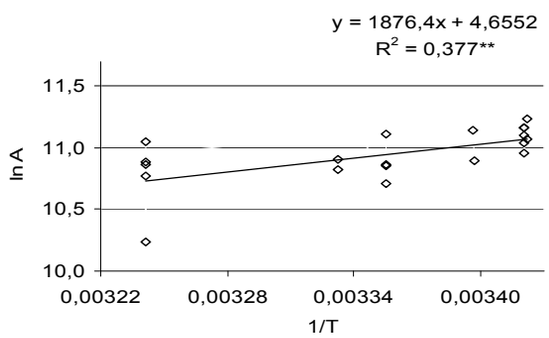

Fig. 4. Regression of ln A (area; bmS) parameter of induction of Chl a fluorescence arameter of induction f induction of Chl a fluorescence and reciprocal values of absolute temperature (1/T; K). **p<0.01.

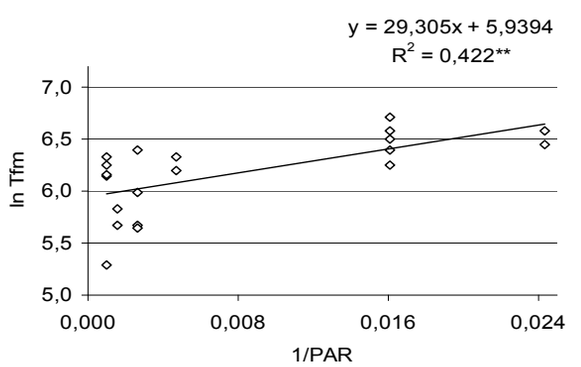

Fig. 5. Regression of ln Tfm (mS) parameter of induction of Chl a fluorescence and reciprocal values of photosyntheticaly active radiation (1/PAR; umol m-2 s-1). **p<0,01.

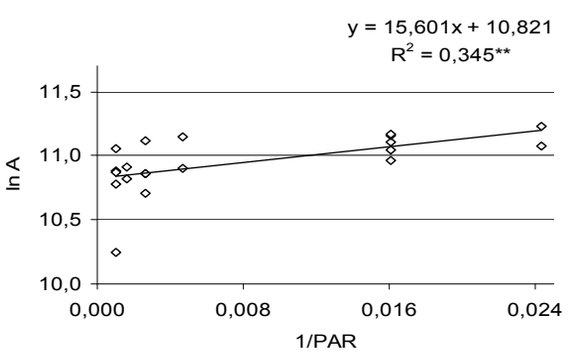

Fig. 6. Regression of ln A (area; bmS) parameter of induction of Chl a fluorescence arameter of induction f induction of Chl a fluorescence and reciprocal values of photosyntheticaly active radiation(1/PAR; umol m-2 s-1). **p<0,01.

m^{-2} s^{-1}); sweet cherry, juniper and barley (PAR≅235 μmol m^{-2} s^{-1}); ribwort plantain, greater plantain, red clover, and white clover (PAR≅400 μmol m^{-2} s^{-1}); common pine and birch (PAR≅800 μmol m^{-2} s^{-1}); T≅20-25°C; altitude ≅1200 m; 200 km south of Belgrade]. Four-replicate measurements were done with dark-acclimated leaf segments (by the so-called leaf fork) over the course of an hour *in situ*.

Abbreviations: PAR) Photosynthetically active radiation; RC PS_2) reaction center of photosystem two; Chl a) chlorophyll a; R/FR acclimation) acclimation of plants to the ratio of red and far red light.

RESULTS AND DISCUSSION

Parameters ln Tfm (Table 1: r=0.649**; Fig. 5: R^2=0,422**) and ln A (Table 1: r= 0,588**; Fig. 6: R^2=0,345**) are the only parameters in highly significant correlation with 1/PAR (μmol m^{-2} s^{-1}). Accordingly, these parameters (under the given conditions in the stated plants) have higher values at lower PAR (and vice versa). This means that light affects the PS_2 activity through changes in the Q_A redox state (ln Tfm) and the capacity of the plastiquinone pool (ln A) in PS_2 (Krause and Weis, 1991), i.e., the acceptor side of PS_2 is affected, as already shown *in vivo* (Tomek et al., 2001). It should be noted that PAR and temperature (under the given conditions) are in highly significant correlation (Table 1). Temperature affects several parameters of Chl a fluorescence. Parameters Fv/Fm (Table 1: r=0,698**; Fig. 1: R^2= 0,487**), Fv/F_0 (Table 1: r= 0,709**; Fig. 2: R^2=0,503**), and ln A (bmS; Table 1: r= 0,614**; Fig. 4: R^2=0,377**) are in highly significant correlation with 1/T, while parameter ln Tfm (mS; Table 1: r =0,490*; Fig. 3: R^2=0,240*) is significantly correlated with 1/T. This means that the lower the temperature (under the given conditions in the stated plants) is, the higher the values of these parameters are and vice versa. Such a result points to complex temperature regulation of PS_2 activity via: a) changes in the quantum yield of PS_2, b) changes of the Q_A redox state, and c) changes in the capacity of the plastiquinone pool in PS_2. Comparing the effects of PAR and temperature on functioning of PS_2, we see that temperature, in contrast to PAR, affects the RC state in PS_2 (Table 1, Figs. 1 and 2). What is the meaning of this? Increase of PAR and temperature raises leaf energy content (Marković et al., 1996), which thermodynamically accelerates different reactions in PS_2 (electron transport, the state of the acceptor side of PS_2, etc.). Due to higher temperatures, thylakoid membrane lipids become more motile, while lower temperatures cause rigidification of these membranes (Lyons et al., 1979; Nishida and Murata, 1996; Marković et al., 1996; Havaux, 1998), which affects the functioning of photosynthetic complexes in the membranes. Various modifications of the state of thylakoid lipids (changes in the role of the xanthophyll cycle: Havaux, 1998; changes in the degree of unsaturation of fatty acids: Nishida and Murata, 1996) affect the degree of membrane reactions when temperatures are changed. Hence, temperature increase lowers the quantum yield in PS_2, at least under the given conditions in the stated plants (Table 1, Figs. 1 and 2), which can be attributed to increased fluidity of the thylakoid membranes (Marković et al., 1996; Havaux, 1998). Furthermore, increased temperatures change the Q_A redox state (ln Tfm; Fig. 3) and the capacity of the plastiquinone pool in PS_2 (ln A; Fig. 4), which means that higher temperatures affect the RC and the state of the acceptor side of PS_2. All this points to different mechanisms by which amplified light and increased temperatures affect photosynthetic processes in the thylakoids. It is our opinion that amplified light directly affects the acceptor side of PS_2 and electron transport in photosynthesis, while high temperatures possibly affect the state of lipids and thereby influence photosynthetic processes in the thylakoids, above all in the RC of PS_2. It is not yet clear whether the mechanisms by which PAR and temperatures affect photosynthetic processes in the thylakoids are individual or complementary. In such a context, it is important to note that the Pindex parameter, as an indicator of total photosynthesis (Strasser et al., 1995), depends on processes in the RC of PS_2 (Fv/Fm, Fv/F_0) and does not depend on processes on the acceptor side of PS_2 (ln Tfm, ln A), at least in these plants under the given ecophysiological conditions (Table 1). It is not yet evident whether the plant species has any significance in the observed processes. Neither PAR nor temperature significantly affect Pindex (Table 1).

Acknowledgments — We wish to thank colleagues from the Institute for Agricultural and Technological Research, Zaječar, for lending us the Handy-PEA fluorometer. Work reported here was supported by grants from the Ministry of Science of the Republic of Serbia.

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УТИЦАЈ ТЕМПЕРАТУРЕ И СВЕТЛОСТИ (PAR) НА ИНДУКЦИЈУ ФЛУОРЕСЦЕНЦИЈЕ CHL A *IN SITU:* ЕКОФИЗИОЛОШКИ ПРЕГЛЕД

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Праћен је утицај PAR и температуре на индукцију флуоресценције Chl a на листовима 20 биљних врста у њиховом природном окружењу. Светлост делује на акцепторску страну PS_2. Регулација активности PS_2 температуром одвија се утицајем на RC и акцепторску страну PS_2. Утицај повишене температуре могуће је објаснити и повећаном флуидношћу тилакоидних мембрана. PAR и температура у датим условима налазе се у високозначајној позитивној корелацији, тако да није јасно

да ли се ради о посебним или комплементарним механизмима дејства PAR и/или темпера-туре на фотосинтетске процесе у тилакоидним мембранама. Такође није јасно да ли специјска специфичност има неки значај у фотосинтетској реакцији биљака на промене PAR и температуре. У овим процесима промене PAR и температуре не утичу на Pindex, као показатељ укупне фотосинтезе.