

Supplement

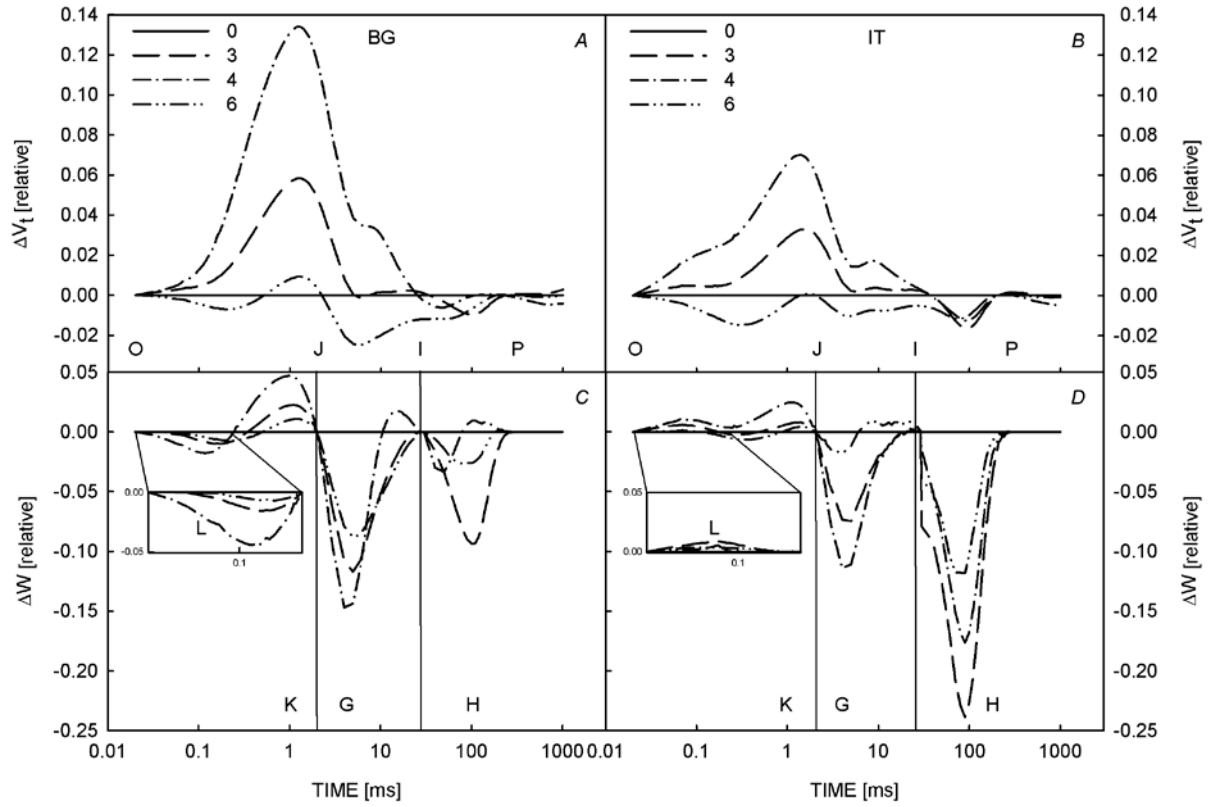


Fig. 1S. Difference curves revealing effects of moderately high temperature on OJIP transients in mature leaves of Bulgarian (BG, Fig. 1SA) and Italian (IT, Fig. 1SB) ecotypes *P. orientalis*. Details in the characteristic L, K, H and G bands are presented on panel C for BG ecotype, and on panel D for IT ecotype plane trees.

Each DC value was calculated by subtracting the values of relative variable fluorescence V_t [$V_t = (F_t - F_0)/(F_M - F_0)$] for mature leaves on day 0 from respective values of V_t for other experimental days, e.g. for Day 3 $\Delta V_t = V_{t(\text{Day } 3)} - V_{t(\text{Day } 0)}$. The presented on the graphics days of the experiment are: Day 0 – control day; Day 3 – third day of the temperature treatment; Day 4 – first day of recovery; and Day 6 – third day of recovery. DC for the specific steps of the induction curve (ΔW) were calculated analogously to the relative variable fluorescence (ΔV_t) for O-P transient, as follow: for O-K phase, L band: $W_{t(\text{OK})} = (F_t - F_0) / (F_K - F_0)$; $\Delta W_{\text{OK}} = W_{t(\text{OK})(\text{day of the experiment})} - W_{t(\text{OK})(\text{Day } 0)}$; for O-J phase, K band: $W_{t(\text{OJ})} = (F_t - F_0) / (F_J - F_0)$; $\Delta W_{\text{OJ}} = W_{t(\text{OJ})(\text{day of the experiment})} - W_{t(\text{OJ})(\text{Day } 0)}$; for J-I phase, H band: $W_{t(\text{JI})} = (F_t - F_J) / (F_I - F_J)$;

$$\Delta W_{JI} = W_{t(JI)(\text{day of the experiment})} - W_{t(JI)(\text{Day 0})}; \text{ for I-P phase, G band: } W_{t(IP)} = (F_t - F_I) / (F_P - F_I);$$

$$\Delta W_{IP} = W_{t(IP)(\text{day of the experiment})} - W_{t(IP)(\text{Day 0})}.$$

The information we obtain from the parameter W_{OJ} is almost the same as with F_K/F_J . We analyzed the correlation between values of the both parameters for the whole experimental data set that we used in our study, and found high Pearson correlation coefficient $R=0.95$, $p=0$, the number of experimental points, $n=1417$. That is why we used the W_{OJ} parameter to evaluate the changes in the chlorophyll fluorescence at the K band. Fig. 2S illustrates the correlation between the both mentioned parameters.

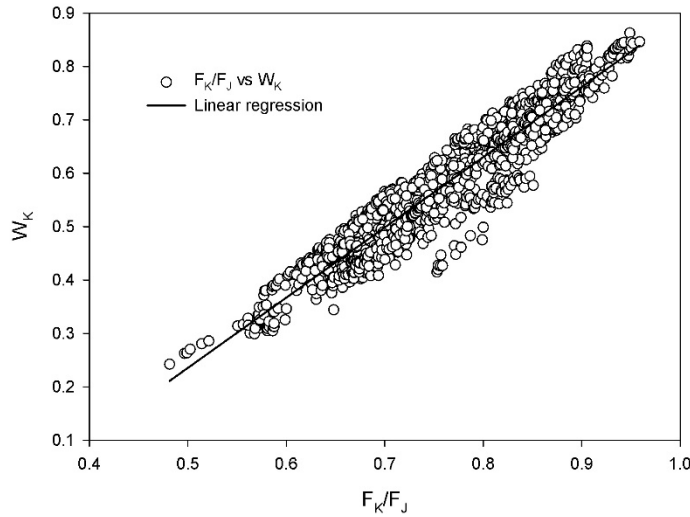


Fig. 2S. Correlation between the parameters W_{OJ} and F_K/F_J .

The correlation between the values of the both parameters for the whole experimental data set was analyzed, and the obtained Pearson correlation coefficient is $R=0.95$, $p=0$, the number of experimental points, $n=1417$.

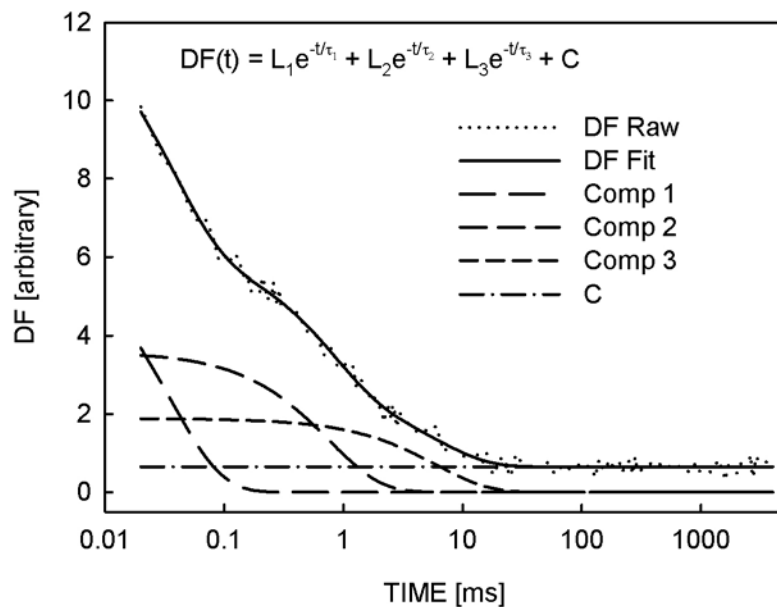


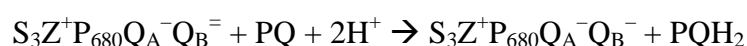
Fig. 3S. DF decay kinetics, recorded after 1 second illumination of mature leaves of *P. orientalis* from Bulgarian ecotype. The DF Raw data presents the curve, recorded by the measuring device. DF Fit is an approximation derived by a 3-exponential fitting model corresponding to the equation at the top of the graph and described in detail below. Decomposition of the curve to different kinetic components is presented. The 4th component is a constant.

DF is a result of backward electron flow and charge recombination in the RC of PSII, which lead to a secondary excitation of the Chl *a* molecules (Goltsev *et al.* 2004, Goltsev *et al.* 2009b). Even though the photosynthetic electron transport has a high efficiency, all redox reactions are reversible and recombination between separated charges in PSII RC is possible. The following secondary excitation of the RC chlorophyll leads to repopulation of the excitations in the antenna Chl molecules and eventually emission of the energy as light quanta, called DF (Zaharieva and Goltsev 2003, Kalaji *et al.* 2014c). Backward electron transport can be generated at every step of the electron transport chain thus giving life to various light emitting precursors. DF is not a result of a single reaction but a sum of many

independent sources of energy excitation, each of them generates different kinetic components of DF, different in their light intensity and lifetime (Li *et al.* 2007). Decay kinetics is considered as a decay curve of multiple overlapping components. Each component is a result of electron recombination in a specific PSII redox state (Markovic *et al.* 2001).

The main PSII redox states able to generate DF are $P_{680}^{+}\text{Pheo}^{-}$, $P_{680}^{+}Q_A^{-}$, $Z^{+}Q_A^{-}$, $S_{i+1}ZQ_A^{-}$, $Z^{+}Q_B^{-}$, and $S_{i+1}ZQ_B^{-}$.

The first DF emitting states are a result of $P_{680}^{+}Q_A^{-}$ recombination and lay in the microsecond range of decay time – 1 μs , 5-10 μs , and 35-40 μs components (Goltsev *et al.* 2009b, a). They reflect the state of PSII electron donor side. Sub-millisecond DF (120-200 μs) is generated by $Z^{+}Q_A^{-}$ state and depends on Q_B redox state – when Q_B is fully oxidized, the electron flow from Q_A has high rates. When Q_B is semi-reduced, Q_B^{-} , this component is observed in 700 μs time region of DF decay. The first millisecond component (1-2-3.5 ms) depends on RCs in S_3 OEC state and later on Q_A^{-} reoxidation while Q_B^{-} become fully reduced (Goltsev *et al.* 2009a). The time course of DF decay at this interval is determined by PQH_2 exchange with oxidized PQ pool:



DF recorded in later periods of time originates from S_2 and S_3 OEC states recombination with Q_A^{-} and Q_B^{-} : $S_2Q_B^{-}$ and $S_3Q_B^{-}$.

The initial part of the DF decay curve can be interpreted mathematically as a sum of exponents (Lavorel 1975). Different components vary by the characteristic time of decay (τ) and the amplitude, L (Christen *et al.* 2000):

$$L(t) = \sum_i L_i \cdot e^{-\frac{t}{\tau_i}}$$

where $L(t)$ is DF, emitted at time t after the switching the illumination off, L_i is the amplitude of the i -component with characteristic lifetime τ_i .

Each decay curve can be presented as a three exponential function:

$$DF(t) = L_1 e^{-t/\tau_1} + L_2 e^{-t/\tau_2} + L_3 e^{-t/\tau_3} + C$$

The constant C introduces all slow components as well as the background electric signal (noise). L_1 , L_2 and L_3 are proportional to the concentrations of the light emitting species and its quantum yield. The characteristic lifetime for each component (τ_1 , τ_2 and τ_3) decreases with $1/e$ and is reversely proportional to the rate constant of the reaction, leading to each state disappearance (Goltsev *et al.* 2004, Goltsev *et al.* 2009b, Kalaji *et al.* 2014b).

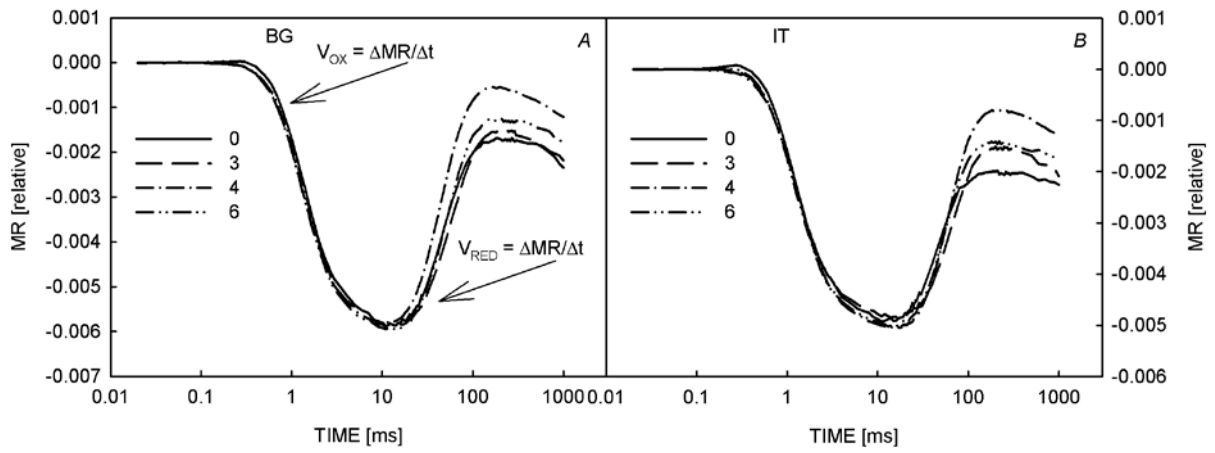


Fig. 4S. Dynamics of the P700 + PC oxidation and re-reduction measured by MR820 signal for Bulgarian (BG, 4SA) and Italian (IT, 4SB) ecotypes are shown for 4 days of the experiment: Day 0 – control day; Day 3 – third day of the temperature treatment; Day 4 – first day of recovery; and Day 6 – third day of recovery. The regions of the curves, where the MR820 signal is practically linearly dependent on time, allow rates of oxidation (V_{OX}) and reduction (V_{RED}) to be derived from maximal slopes of the MR820 curves approximately at points, indicated by arrows.