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BRIEF COMMUNICATION

Estimation of changes in the activity of photosynthetic apparatus of plant leaves based on half-time of fluorescence intensity decrease

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Abstract

The range of variations in parameter $\tau_{0.5}$ – half-time of fluorescence intensity decrease during the slow phase of chlorophyll fluorescence induction (CFI) – has been studied during ontogeny of leaves of monocotyledonous and dicotyledonous plants in plant communities of different structures. Plants were grown hydroponically on expanded clay aggregate in growth chambers, under PPFD of 400 μmol(photon) m^{-2} s^{-1}, under controlled conditions. Analysis of the literature data and results of experimental observations of $\tau_{0.5}$ behavior compared to other CFI parameters, nonphotochemical quenching coefficient, q_N, in particular, leads to the conclusion that parameter $\tau_{0.5}$ can be effectively used for indirect estimation of variations in the activity of photosynthetic apparatus during ontogeny of plant leaves.

Additional key words: chlorophyll fluorescence induction; leaf age; photosystem II.

Dramatic global climate change is a factor that increases the importance of stress tolerance of plants, on the one hand, and urges researchers to develop methods for monitoring the state of the plants, on the other. All stressors, whatever part of the plant they may affect, eventually, directly or indirectly, influence photosynthesis, which is primarily related to the activity of photosynthetic apparatus (PSA) at the leaf level. In recent years, the method of chlorophyll fluorescence induction (CFI) based on the Kautsky effect has become a widespread method for ecological monitoring of plant communities (Lichtenthaler *et al.* 2005, Lazár 2015, Kalaji *et al.* 2017). Sometimes, when plants are subjected to external stresses, it is essential to determine quickly the functional state of the leaf PSA and estimate reversibility (or extent) of changes in PSA activity. A common approach is to use a set of characteristics based on measurements of amplitude parameters of the plant leaf CFI curve (Roháček 2002).

An important and frequently used indicator of quantum efficiency of photosynthesis is the ratio of variable to maximum fluorescence: F_v/F_m, where $F_v = F_m - F_0$, and maximum F_m is measured at the moment of the powerful flash saturating photosynthesis (Lazár 2015, Kalaji *et al.* 2017). An alternative quantitative characteristic of photosynthesis is fluorescence decrease index $R_{fd} = (F_P - F_S)/F_S$, also known as vitality index, where F_P is maximum (within the photoinduction curve) chlorophyll (Chl) fluorescence under nonsaturating light conditions and F_s is steady-state fluorescence level (Lichtenthaler *et al.* 2005). R_{fd} is more sensitive to external impacts than F_v/F_m, and this value correlates well with the specific rate of photosynthetic fixation of CO_2 in normal green tissues (Lichtenthaler and Rinderle 1988, Lichtenthaler *et al.* 2005). However, changes in the values of parameters R_{fd} and F_v/F_m are not helpful in estimating the degrees of irreversible changes in PSA caused by ageing and stress (Lichtenthaler and Rinderle 1988, Venedictov *et al.* 1999). Parameters of the slow phase (the so called P-S-M-T phase) of CFI may be particularly useful for understanding the mechanisms of PSA stability under extreme conditions. This phase of CFI is very closely related to biochemical processes of photosynthesis and to CO_2 gas exchange at the level of plant leaves (Lichtenthaler and Rinderle 1988, Kalaji *et al.* 2017). At the present time, there is no generally accepted theoretical description of slow phase curves. One of the reasons for this is that it is very difficult

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Abbreviations: AL – actinic light intensity; Chl – chlorophyll; CFI – chlorophyll fluorescence induction; ETR – electron transport rate; F_P – maximal fluorescence at peak P; F_S – steady-state fluorescence level; F_p/F_s – the fluorescence at peak level P (F_p) to steady -state fluorescence (F_s) ratio; F_v/F_m – maximum quantum yield of PSII photochemistry; NPQ – nonphotochemical quenching; PSA – photosynthetic apparatus; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; R_{fd} – "vitality index" (relative fluorescence decrease ratio); Φ_{PSII} – effective quantum yield of PSII photochemistry; $\tau_{0.5}$ – half-time of fluorescence intensity decrease is time, when variable fluorescence during the slow phase of CFI $F_d = F_p - F_s$ decreases to half of its value.

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to model the dynamics of slow processes influencing CFI, such as generation of transmembrane electrochemical potential, interaction between the primary reactions of photosynthesis and the carbon fixation cycle, membrane energization, chloroplast and mitochondrial respiration, *etc.* (Semihatova 1992, Riznichenko *et al.* 2009). A number of studies relating properties of the curves of slow CFI to component concentrations and constants of rates for certain processes have been published recently (Riznichenko *et al.* 2009, Kirzhanov and Kukushkin 2010, Lyu and Lazár 2017a,b).

Two questions arise: Can be a state of PSA evaluated by using a minimal set of fluorescent parameters and what properties must these parameters have? It would appear that (*1*) the value of a parameter included in this set must not vary too much during ontogeny of the leaf, at least between the time of leaf formation and early senescence; (*2*) the parameter must remain sufficiently stable under widely varying fluorescence-exciting light – actinic light (AL) – intensity (Nesterenko *et al.* 2012, 2015); and (*3*) the parameter must be sensitive to changes in PSA activity under stress and recovery of the PSA state once the stress ceases (Li *et al.* 2013, Shikhov *et al.* 2016).

Nesterenko *et al.* (2014, 2015) studied age dependence of the main characteristics of CFI and the effect of AL intensity on their values in wheat (*Triticum aestivum* L.) leaves. The authors determined such parameters as quantum yield in PSII (F_v/F_m), effective quantum yield in PSII (Φ_{PSII}); q_P, q_N, and NPQ – parameters of photochemical and nonphotochemical quenching of Chl fluorescence; the F_P/F_t ratio; and vitality index R_{fd}. At AL intensity about 380 μmol(photon) m^{-2} s^{-1}, the effect of the leaf age on fluorescent parameters was insignificant, except the effect on R_{fd}. An increase in AL intensity to 580 μmol(photon) m^{-2} s^{-1} and higher caused a considerable age dependence of fluorescent parameters. The F_P/F_S ratio and parameters of nonphotochemical quenching of fluorescence – NPQ and q_N – were found to be the most sensitive to the leaf age (Hong *et al.* 2000, Nesterenko *et al.* 2015). These fluorescent parameters are often used to estimate PSA stress tolerance (Lichtenthaler *et al.* 2005, Shikhov *et al.* 2011, Ashraf 2013). The most commonly studied parameters are changes in fluorescence at peak level P (F_P), steady-state fluorescence (F_S), their ratio, R_{fd} (Lichtenthaler and Rinderle 1988, Šesták and Šiffel 1997, Nesterenko *et al.* 2006), and other properties of PSII that are determined from the basic recorded amplitude parameters (Lazár 2015, Kalaji *et al.* 2017). However, external impacts, including light, may change not only amplitude parameters of CFI, which are related to intensities of the processes at different levels of organization of PSA, but also temporal parameters of these processes (Lichtenthaler and Rinderle 1988, Nesterenko and Siďko 1993, Kharuk *et al.* 1994, Bukhov 2004). Thus, temporal parameters of CFI deserve special attention. The simplest of them is $\tau_{0.5}$ – half-time of fluorescence intensity decrease during the slow phase of Chl fluorescence induction (CFI) (Nesterenko and Siďko 1993, Šesták and Šiffel 1997). The declining CFI curve reflects a number of photosynthetic processes: (*a*) enhancement of the non-photochemical quenching of fluorescence, caused by an increase in the dissipation of absorbed energy in PSII, (*b*) activation of the Calvin–Benson cycle, leading to a faster rate of electron outflow from PSI, (*c*) redistribution of the light-collecting antenna system between PSII and PSI, *etc.* (Korneev 2002, Riznichenko *et al.* 2009, Kalaji *et al.* 2017). Slow changes in Chl fluorescence are not only related to the photochemical use of light but they also carry information on mechanisms involved in PSA protection against damage caused by radicals (Bukhov 2004, Jahns and Holzwarth 2012). Kinetics of fluorescence intensity decrease in the light is often nonmonotonic, and, thus, it cannot be described by a sum of exponential curves. Therefore, we and other researchers used a formal parameter – $\tau_{0.5}$, an integrated parameter of the activation rates of certain photoassimilative and photoprotective processes in the leaf, as a temporal parameter of the CFI curve (Nesterenko and Siďko 1993, Kharuk *et al.* 1994, Šesták and Šiffel 1997, Nesterenko *et al.* 2012, Ptushenko *et al.* 2013).

A previous study showed that an increase in the intensity of the fluorescence-exciting light from 20 to 80 W m^{-2} [PPFD from 90 to 370 μmol(photon) m^{-2} s^{-1}] did not cause any significant changes in the $\tau_{0.5}$ parameter of the CFI curves for a mature 18-d-old radish leaf (Nesterenko *et al.* 2012). Analysis of the age dependence of $\tau_{0.5}$ showed that it remained relatively stable over a certain period of time, which corresponded to the maturity stage of the leaf (Kharuk *et al.* 1994, Šesták and Šiffel 1997, Nesterenko *et al.* 2006, 2012). However, no data on the range of $\tau_{0.5}$ variation under conditions, which are optimal for plant growth and development, can be found in literature. Thus, the purpose of this study was to determine the range of variation in $\tau_{0.5}$ of CFI curves during ontogeny of the leaves of monocotyledonous (wheat) and dicotyledonous (cucumber and radish) plants in plant communities of different structures under controlled artificial light.

Samples of variously aged wheat (*Triticum aestivum L.* cv. 232, selected by Lisovsky (Tikhomirov *et al.* 1991) leaves of the fifth tier counted from the stem base; the third leaves of radish (*Raphanus sativus* L. var. *radicula* cv. Virovskii White); and cucumber (*Cucumis sativus L.* cv. Moskovskii Teplichnyi) leaves of the third tier were used in this study. Plants were grown hydroponically on expanded clay aggregate, in growth chambers, under PPFD of 400 μmol(photon) m^{-2} s^{-1}. This light intensity has been found to be the most favorable for achieving the highest photosynthetic efficiency of plant communities (Tikhomirov and Siďko 1982, Tikhomirov *et al.* 1991). Wheat and radish plants were grown under continuous light; cucumber plants were grown with a photoperiod of 14-h light/10-h dark. The air temperature was maintained at 20 ± 1°C, CO_2 varied between 0.3–0.8%, and relative humidity was 70–80%. Experiments were run in triplicate. CFI curves were recorded using a single-beam instrument (Nesterenko *et al.* 2012). The time of recording of a CFI curve was 3–5 min, and the time of dark adaptation was 30 min. For estimating the physiological state of the plant photosynthetic apparatus, we chose two parameters: the F_P/F_S ratio and $\tau_{0.5}$ – half-time of fluorescence intensity decrease, which is equal to the time needed to achieve half of the value of variable fluorescence $F_d = F_P - F_S$ observed over the time period with lights on (3–5 min) (Kharuk *et al.* 1994, Šesták and Šiffel 1997). The approaching of F_S was determined visually. Parameter of F_P/F_S, like a similar parameter $R_{fd} = F_P/F_S - 1$ ("vitality index"), characterizes photosynthetic activity of leaves (Lichtenthaler and Rinderle 1988, Karavaev and Polyakova 1998).

Fig. 1 shows characteristic curves representing the slow phase of CFI in variously aged leaves of wheat, cucumber, and radish plants grown under continuous light of 400 μmol(photon) m^{-2} s^{-1}. The kinetics of fluorescence intensity decrease in the light was determined by the age of the leaf and showed a nonmonotonic pattern. Changes of the shapes of fluorescence induction curves during ontogeny of plant leaves were described in more detail elsewhere (Šesták and Šiffel 1997, Nesterenko *et al.* 2006).

Analysis of the age dependence of CFI parameters (Table 1) suggested that for some time period, their values remained relatively stable. Such period corresponded to the period of leaf maturity, but its duration differed between plant species, which is supported by the literature (Kharuk *et al.* 1994, Šesták and Šiffel 1997, Nesterenko and Tikhomirov 2005).

Experiments showed that $\tau_{0.5}$ values for nearly mature leaves [80–90% of the maximum leaf area by Mokronosov (1981)] were similar to each other and did not change significantly during ontogeny, between the time of the leaf formation and senescence (Table 1). For three plant species, variations in $\tau_{0.5}$ values were no more than 80% over about two weeks (between day 6–7 and 20–22, Table 1). The $\tau_{0.5}$ values only increased in senescent leaves, *e.g.*, in 25-d-old wheat leaf of the fifth tier, the $\tau_{0.5}$ value more than doubled compared with mature (16–20-d-old) leaves. In a study by Nesterenko *et al.* (2012), the $\tau_{0.5}$ value for a mature radish leaf remained rather stable, varying by no more than 26%, under widely varied intensity of the fluorescence-exciting light. At the same time, $\tau_{0.5}$ was sensitive to changes in the state of PSA under stress. As reported in the study by Shikhov *et al.* (2016), under mild drought conditions, at the third day of the experiment, the $\tau_{0.5}$ value for lettuce increased 2.4-fold, but the parameter recovered to its previous value quicker than other PSA parameters (such as effective quantum yield of PSII – Φ_{PSII}, ETR) when irrigation of plants was resumed (Shikhov *et al.* 2016).

In a study by Ptushenko *et al.* (2013), the $\tau_{0.5}$ in the leaves of mountain ash changed nonmonotonically with the increasing distance between the trees and the road. As this distance became greater, the $\tau_{0.5}$ increased, reaching its highest value, about 20–30 s, at 50 m away from the crossroads, but decreased to 10 s at greater distances. The authors of that paper suggest that the nonmonotonic dependence of $\tau_{0.5}$ on the distance between the trees and the road indicates that adaptation of the photosynthetic apparatus of plants to environmental conditions is multifactorial.

An explanation proposed by the authors (Ptushenko *et al.* 2013) is that time dependence of the curve of Chl fluorescence induction is determined by some interrelated factors: the state transitions (state 1 – state 2), the cyclic and/or alternative electron transport routes, effects of the activation kinetics of ferredoxin NADP$^+$ reductase and then the activation of enzymes of the Calvin Benson cycle.

Table 1. Variations in the CFI parameter $\tau_{0.5}$ during ontogeny of wheat, cucumber, and radish leaves. The data in the table are arithmetic means ± standard error, *n* = 4.

Plants	Leaf age [d]	Leaf area [%]	$\tau_{0.5}$, [s]	F_P/F_S [rel. u.]
Wheat	2	20	9.1 ± 1.1	3.0 ± 0.2
	7	80	10.1 ± 1.7	3.5 ± 0.1
	16	90	12.3 ± 1.1	2.6 ± 0.1
	20	100	16.2 ± 1.2	2.4 ± 0.2
	25	100	35.3 ± 1.8	2.0 ± 0.3
Cucumber	5	50	12.4 ± 1.3	3.0 ± 0.4
	7	75	8.2 ± 1.2	3.5 ± 0.2
	12	85	12.4 ± 1.3	3.2 ± 0.3
	14	90	14.4 ± 1.6	2.4 ± 0.2
	22	100	14.6 ± 1.8	1.9 ± 0.5
	30	100	22.3 ± 2.5	1.3 ± 0.5
Radish	6	50	12.3 ± 1.5	3.3 ± 0.2
	12	90	14.4 ± 1.3	2.0 ± 0.1
	16	97	14.6 ± 1.5	1.6 ± 0.1
	24	100	20.5 ± 1.0	1.4 ± 0.2

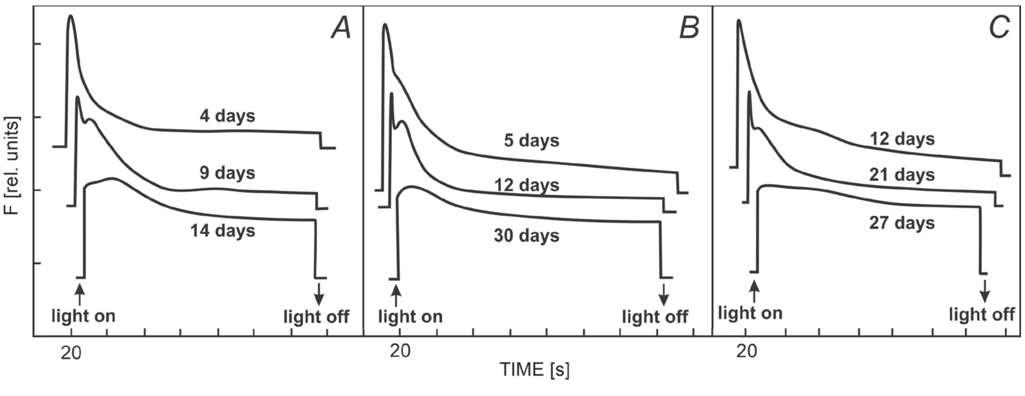

Fig. 1. Characteristic curves representing the slow phase of CFI in variously aged leaves of wheat (*A*), cucumber (*B*), and radish (*C*) plants grown under continuous light at a light intensity of 400 μmol(photon) m^{-2} s^{-1} PPFD. The dates at the curves denote the age of the leaves.

which are under control of the thioredoxin system that determine the rate of electron outflow from PSI, and the nonphotochemical quenching of fluorescence (Ptushenko *et al.* 2013).

Comparison of Chl fluorescence parameters of two sugar beet species with different PSA tolerance to drought showed different responses of parameters F_0, F_v/F_m, q_P, and q_N to this stress (Li *et al.* 2013). Of these PSA parameters, nonphotochemical quenching coefficient, q_N, showed the highest sensitivity to water stress (by varying almost threefold) (Li *et al.* 2013). During ontogeny of wheat plants, this parameter varied within 30%, which was comparable with variations in $\tau_{0.5}$ (Table 1). Parameters q_N and $\tau_{0.5}$ are comparable in their changes during ontogeny and, according to the literature, sensitivity to stresses (Li *et al.* 2013, Ptushenko *et al.* 2013, Shikhov *et al.* 2016), but the advantage of $\tau_{0.5}$ is that it is simpler to measure. In order to determine q_N, one needs to measure four parameters using a PAM fluorometer, while $\tau_{0.5}$ determination is based on only two parameters – F_P and F_S – measured by using a simpler conventional instrument (Korneev 2002, Ptushenko *et al.* 2013).

Chl fluorescence can serve as an indicator of stress, but it cannot differentiate between the types of stress, as many stress factors produce similar effects on the PSII activity (Lichtenthaler and Rinderle 1988, Karavaev and Polyakova 1998, Fracheboud and Leipner 2003, Ashraf and Harris 2013). Some authors consider plant ageing as a stress (with endogenous changes impairing the internal environment) (Thomas *et al.* 2003). At the same time, for chloroplasts, light is regarded as both a photosynthesis substrate and a stressful factor (Bukhov 2004). Thus, leaf age dependence of fluorescent parameters obtained in this study (Table 1), literature data on the effect of the intensity of fluorescence-exciting light (Nesterenko *et al.* 2012), and the use of temporal parameter $\tau_{0.5}$ for monitoring air pollution (Ptushenko *et al.* 2013) and minor drought (Shikhov *et al.* 2016) suggest that this is a promising parameter for estimating PSA activity under stress. Further research is needed, however, to establish quantitative relationships between PSA activity and fluorescent parameter $\tau_{0.5}$ under different stresses.

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