DOI: 10.1007/s11099-015-0133-2 PHOTOSYNTHETICA 53 (4): 617-620, 2015

BRIEF COMMUNICATION

# Effect of light intensity on the age dependence of nonphotochemical fluorescence quenching in wheat leaf

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## Abstract

The effects of actinic light (AL) intensity on the age dependence of nonphotochemical fluorescence quenching ($q_N$) and effective quantum yield in PSII ($\Phi_{PSII}$) were studied in continuously illuminated wheat leaves of the upper tier. Regular changes were revealed in both age dependence of $q_N$ at elevated AL intensities and light curves of $q_N$. These changes are related to alterations in strategies of redistribution and use of absorbed light energy by the photosynthetic apparatus at different stages of wheat leaf development. Unlike $\Phi_{PSII}$, $q_N$ as a parameter was more sensitive to the differences in the leaf age at a certain range of light intensities. At the same time, the stability of $q_N$ at moderate light intensities may serve as an indication of leaf maturity.

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

Nonphotochemical quenching of fluorescence ($q_N$) is commonly used to analyze stress tolerance in plants (Baker 2008, Ashraf and Harris 2013). It is well known that $q_N$ involves at least three molecular mechanisms: ΔpH-dependent quenching, which determines energy-dependent quenching of fluorescence ($q_E$ or the fast component, $q_{Nf}$); transition of the pigment system from state 1 to state 2 ($q_T$ or the medium component, $q_{Nm}$); and photoinactivation of PSII ($q_I$ or the slow component, $q_{Ns}$) (Baker 2008). The contribution of each component to the total $q_N$ is determined by the actinic light intensity (Bukhov 2004); their proportions and significance change during the leaf ontogeny (Hong *et al.* 1999, Müller *et al.* 2001), which may affect age variations in the $q_N$ as a whole. Indeed, under low and medium intensities of actinic light (AL), differences in the $q_N$ between mature and aged leaves are insignificant. These differences, however, become greater as AL intensity increases (Hong *et al.* 1999). Mechanisms determining $q_N$ of excited states of chlorophyll (Chl) are mainly studied for PSII, as this photosystem is considered to be more sensitive to photoinhibition than PSI. Although it is well known that $q_N$ is age- and light-dependent, the age dependence of $q_N$ under medium intensities of AL and the effect of an increase in the AL intensity have not been sufficiently studied. No data have been reported on light curves of $q_N$ for leaves of different ages. The AL intensities reported in the literature varied between 200 and 1,500 μmol $m^{-2}$ $s^{-1}$ PPFD (Hong *et al.* 1999, Lichtenthaler *et al.* 2005, Wang and Chen 2011) and the available experimental data are not sufficient to evaluate the effect of the AL on the age dependence of the $q_N$ in the plant leaves. Thus, the purpose of this experiment was to study more thoroughly the age dependence of $q_N$ and the influence of the AL intensity. This study revealed regular changes in both age dependence of $q_N$ at elevated AL intensities and light curves of $q_N$, which are related to alterations in strategies of redistribution and use of absorbed light energy by the photosynthetic apparatus at different stages of wheat leaf development.

*Received* 17 March 2014, accepted 29 January 2015.
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*Abbreviations*: AL – actinic light; CFI – chlorophyll fluorescence induction; Chl – chlorophyll; $F_0$ – minimal fluorescence yield of the dark-adapted state; $F_0'$ – minimal fluorescence yield of the light-adapted state; $F_m$ – maximal fluorescence yield of the dark-adapted state; $F_m'$ – maximal fluorescence yield of the light-adapted state; $F_s$ – steady-state fluorescence yield; $F_v$ – variable fluorescence; $F_v/F_m$ – maximum quantum yield of PSII photochemistry; $q_N$ – nonphotochemical quenching; $\Phi_{PSII}$ – effective quantum yield of PSII photochemistry.
*Acknowledgements*: The study was performed within the framework of subject No. 56.1.4., Section VI in accordance with State Program of RAS Fundamental Research for 2013–2020.

Wheat (*Triticum sativus* L. cv. 232, selected by G.M. Lisovsky) plants were used in this study. Plants were grown in the growth chamber under controlled conditions, under continuous light of 600 μmol $m^{-2}$ $s^{-1}$ PPFD, following the procedure described by Tikhomirov and Sid'ko (1982). The temperature of the air was maintained at 24 ± 1°C. Relative humidity was 65–75%. Chl fluorescence induction (CFI) was measured in the 2- to 16-d-old wheat leaves of the fifth tier (counted from the stem base and numbered in the order they emerged). Measurements were taken on the middle part of the leaf. The leaves were not detached from the plant during the measurements. The time between the start of illumination and the measurement was 5 min. At the beginning of the measurements, the plants were 16 d old. Five age points corresponded to different stages of leaf development: 2- and 5-d-old leaves – young leaves; 9-d-old leaves – mature leaves; 12- and 16-d-old leaves – aged leaves (Nesterenko *et al*. 2014). Measurements of CFI parameters were performed with a *PAM-2100* fluorometer (*Heinz Walz*, Germany) at irradiances of 380, 575, 810, and 1,330 μmol $m^{-2}$ $s^{-1}$ PPFD. Before measurements, the leaves were dark-adapted for 30 min. The $q_N$ and other parameters [the maximum quantum yield of PSII photochemistry ($F_v/F_m$) and the effective quantum yield of PSII photochemistry ($\Phi_{PSII}$)] were calculated from the following formulas: $q_N = (F_v - F_v')/F_v$, $\Phi_{PSII} = (F_m' - F_t)/F_m'$ (Lichtenthaler *et al*. 2005). At a given PPFD, $\Phi_{PSII}$ provides an estimate of the quantum yield of linear electron flux through PSII. All of the measurements were performed four times for different leaves. The new set of leaves was selected for each time point and the means and standard error (SE) were calculated.

The $F_v/F_m$ values in the 2- to 16-day-old leaves of the fifth tier varied between 0.80 and 0.82. Age variations of $\Phi_{PSII}$ for each AL intensity were as follows: 8, 24, 9, and 24% for AL of 380, 575, 810, and 1,330 μmol $m^{-2}$ $s^{-1}$ PPFD (Fig. 1*A*). After the leaves reached maturity (9 d), this parameter changed insignificantly. At the same time, as the AL intensity was increased, the curves of age dependence of this parameter became lower. The smallest differences in $\Phi_{PSII}$ were observed for mature 9-d-old leaves under adjacent irradiances: 380 and 575 μmol $m^{-2}$ $s^{-1}$ PPFD (Fig. 1*A*). The age dependence of $q_N$ was essentially

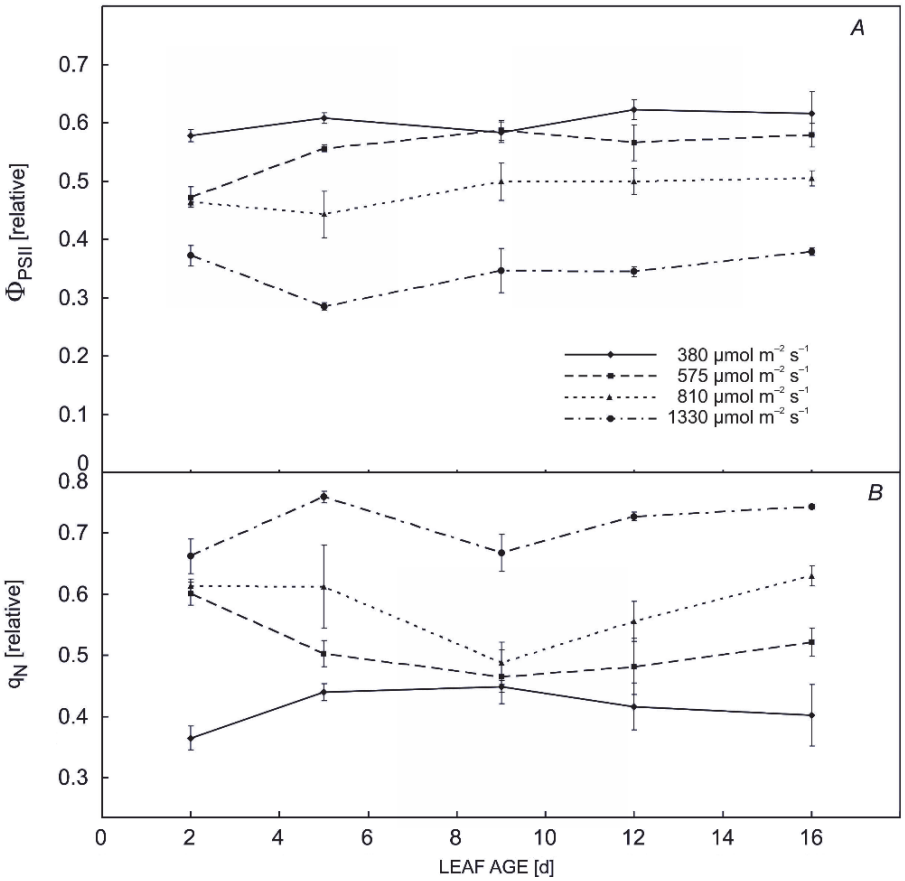


Fig. 1. Age dependence of chlorophyll fluorescence parameters: effective quantum yield in PSII photochemistry, $\Phi_{PSII}$ (*A*) and nonphotochemical quenching of variable chlorophyll fluorescence, $q_N$ (*B*) in wheat (*Triticum sativus* L.) leaves of the fifth tier under different actinic light intensities: 380; 575; 810; 1,330 μmol $m^{-2}$ $s^{-1}$ PPFD. Bars indicate SE ($n$ = 4). Means are shown.

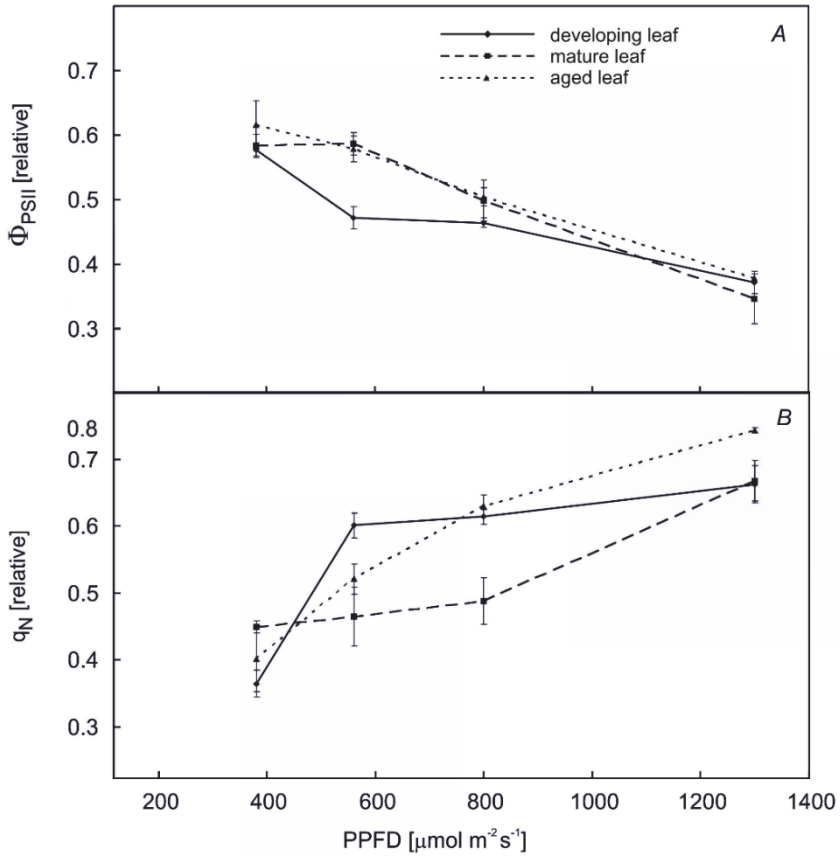


Fig. 2. Age dependence of chlorophyll fluorescence parameters: effective quantum yield in PSII photochemistry, $\Phi_{PSII}$ (*A*) and nonphotochemical quenching of variable chlorophyll fluorescence, $q_N$ (*B*) in variously aged wheat (*Triticum sativus* L.) leaves of the fifth tier. Bars indicate SE ($n$ = 4). Means are shown.

different from that of $\Phi_{PSII}$ as the AL intensity increased (Fig. 1*B*). In mature, 9-d-old leaves, $q_N$ remained almost unchanged under irradiances between 380 and 810 μmol $m^{-2}$ $s^{-1}$ (Fig. 1*B*). It is well known that in mature leaves exposed to certain external factors, many of the parameters remain stable (Wang *et al.* 2011, Nesterenko *et al.* 2012, 2014), as all components of the photosynthetic apparatus of mature leaves retain their coordinated function under a wide range of irradiances. The high stability of photosynthesis under various light conditions is based on a combination of dynamic regulatory light and dark reactions of plants (Bukhov 2004). For younger and older leaves, $q_N$ increased as the light intensity became higher. Age-dependent changes in $q_N$ under AL intensities between 380 and 575 μmol $m^{-2}$ $s^{-1}$ were about 23%, while at 820 and 1,330 μmol $m^{-2}$ $s^{-1}$ they reached 21 and 14%, respectively (Fig. 1*B*). The $\Phi_{PSII}$-light curves for the mature and aged leaves (Fig. 2*A*) overlapped, but the curve for the developing leaf was positioned much lower (Fig. 2*A*). For the mature and aged leaves, between 380 and 575 μmol $m^{-2}$ $s^{-1}$ PPFD, $\Phi_{PSII}$ remained high, but then gradually decreased. All three curves tended to converge at 380 μmol $m^{-2}$ $s^{-1}$ PPFD. For leaves of different ages, the $q_N$-light curves differed more considerably than the $\Phi_{PSII}$-light curves (Fig. 2*B*). Between 380 and 810 μmol $m^{-2}$ $s^{-1}$ PPFD, $q_N$ for the mature leaf (Fig. 2*B*) changed very little, while for the developing and aged leaves (Fig. 2*B*), $q_N$ was growing from the beginning of the light curve. Convergence of the $q_N$-light curves was observed at 380–450 μmol $m^{-2}$ $s^{-1}$ PPFD. As the AL intensity changed from 380 to 1,330 μmol $m^{-2}$ $s^{-1}$ PPFD, the age dependence of $\Phi_{PSII}$ values was insignificant; $\Phi_{PSII}$ were equal to 36–41% of the values at 380 μmol $m^{-2}$ $s^{-1}$ PPFD (Fig. 2*A*). At the same time, these variations in the $q_N$ values were about 49% for the mature leaf and 82 and 85% (1.7 times higher) for the developing and aged leaves, respectively (Fig. 2*B*).

With their age, leaves changed the strategy of distribution and use of absorbed light energy. In the young leaves, the primary function is a rapid growth, which requires sufficient amounts of assimilates, and, thus, photochemical conversion prevails. Aged leaves, where the photoprotective function is more important, utilize excess light energy and accumulate and store it (Bukhov 1997, Hong *et al.* 1999). As reported by Chermnykh and Kosobrukhov (1987), the photosynthetic rate increased only at light intensities no higher than 400 μmol $m^{-2}$ $s^{-1}$ PPFD. As light intensity increased further, $CO_2$

assimilation declined although the rate of ribulose-1,5-bisphosphate carboxylation was relatively high. It has been suggested that the rate of net photosynthesis per unit of area for individual leaves increases with increasing irradiation, reaching saturation at 600 and 900 μmol $m^{-2}$ $s^{-1}$ PPFD for $C_3$ and $C_4$ plants, respectively (Loomis and Amthor 1999).

Nesterenko and Tikhomirov (2005) showed that the choice of the plant developmental stage determines the interpretation of CFI parameters for evaluating the state of the photosynthetic apparatus. This is particularly important in the case of a long-term exposure of plants to unfavorable factors, as accelerated aging is very often the only response of the photosynthetic apparatus to such exposure (Nesterenko *et al.* 2003, 2006). Then, determination of the starting point for leaf senescence may be a way how to evaluate a resistance of the photosynthetic apparatus to the stress factors (Nesterenko *et al.* 2006). As the age heterogeneity of plant leaves usually exists, one should take into account the influence of the leaf developmental stage on the $q_N$-light curves, especially when this parameter is used to study the response of the photosynthetic apparatus to stress.

Further studies are needed to investigate the influence of different illumination on the choice of the optimal AL intensity for measuring $q_N$ in leaves. Even now, however, analysis of the literature data (Chermnykh and Kosobrukhov 1987, Loomis *et al.* 1999, Bukhov 2004) and our previous results (Nesterenko *et al.* 2012, 2014) suggested that for $C_3$ plants the AL intensity for measuring $q_N$ should not be higher than 400 μmol $m^{-2}$ $s^{-1}$ PPFD in order to reduce the effect of the age heterogeneity of leaves. The stability of $q_N$ under AL intensities between 400 and 600 μmol $m^{-2}$ $s^{-1}$ PPFD may be considered as an indication of leaf maturity. However, for other species/ cultivars and growth conditions this range of light intensities should be determined experimentally.

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