PHOTOSYNTHETICA 40 (1): 139-144, 2002

Relationship between photosystem 2 electron transport and photosynthetic CO_2 assimilation responses to irradiance in young apple tree leaves

JIA Husen[*,***] and LI Dequan[*,****]

College of Life Sciences, Shandong Agricultural University, Shandong Tai'an 271018, China[*]
Laboratory of Photosynthesis Basic Research, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China[**]

Abstract

The responses to irradiance of photosynthetic CO_2 assimilation and photosystem 2 (PS2) electron transport were simultaneously studied by gas exchange and chlorophyll (Chl) fluorescence measurement in two-year-old apple tree leaves (*Malus pumila* Mill. cv. Tengmu No.1/*Malus hupehensis* Rehd). Net photosynthetic rate (P_N) was saturated at photosynthetic photon flux density (PPFD) 600-1 100 μmol m^{-2} s^{-1}, while the PS2 non-cyclic electron transport (*P*-rate) showed a maximum at PPFD 800 μmol m^{-2} s^{-1}. With PPFD increasing, either leaf potential photosynthetic CO_2 assimilation activity (F_d/F_s) and PS2 maximal photochemical activity (F_v/F_m) decreased or the ratio of the inactive PS2 reaction centres (RC) $[(F_i - F_0)/(F_m - F_0)]$ and the slow relaxing non-photochemical Chl fluorescence quenching (q_s) increased from PPFD 1 200 μmol m^{-2} s^{-1}, but cyclic electron transport around photosystem 1 (RFp), irradiance induced PS2 RC closure $[(F_s - F_0')/(F_m' - F_0')]$, and the fast and medium relaxing non-photochemical Chl fluorescence quenching (q_f and q_m) increased remarkably from PPFD 900 μmol m^{-2} s^{-1}. Hence leaf photosynthesis of young apple leaves saturated at PPFD 800 μmol m^{-2} s^{-1} and photoinhibition occurred above PPFD 900 μmol m^{-2} s^{-1}. During the photoinhibition at different irradiances, young apple tree leaves could dissipate excess photons mainly by energy quenching and state transition mechanisms at PPFD 900-1 100 μmol m^{-2} s^{-1}, but photosynthetic apparatus damage was unavoidable from PPFD 1 200 μmol m^{-2} s^{-1}. We propose that Chl fluorescence parameter *P*-rate is superior to the gas exchange parameter P_N and the Chl fluorescence parameter F_v/F_m as a definition of saturation irradiance and photoinhibition of plant leaves.

Additional key words: chlorophyll fluorescence; *Malus*; net photosynthetic rate; photosystem 2; quenching.

Introduction

Photosynthetically active radiation is the energy source of plant photosynthesis. At low irradiance, photosynthetic rate is a linear function of irradiance. With increasing irradiance photosynthesis becomes saturated, and finally, at high irradiances, inhibited. This is the common photon response characteristic of plant photosynthesis (Prezelin 1981, Falkowski *et al.* 1994, Iqbal *et al.* 1997). During photosynthesis, photon energy absorbed by plant is converted into chemical forms, NADPH and ATP, and then used for CO_2 assimilation. When too many photons are absorbed by leaves, excess NADPH and ATP are produced in photochemical reactions which causes a decrease in leaf photosynthetic capacity (Powles *et al.* 1979, Krall and Edwards 1992): this is called photoinhibition (Kok 1956, Jones and Kok 1966).

The ability of photon energy conversion in plant is mainly reflected in photochemical reactions of photosynthesis, which can be determined by the non-invasive Chl fluorescence measurement. Schindler and Lichtenthaler (1996) emphasised that the Chl fluorescence signals measured at the upper leaf side are not representative of the chloroplasts of whole leaf, and thus differ from P_N measured by gas exchange. P_N reflects photosynthetic characteristics of whole leaf (Schindler and Lichtenthaler

Received 19 November 2001, *accepted* 25 March 2002.
[***]Author for correspondence; fax: +86 538 8249608; e-mail: dqli@sdau.edu.cn
Abbreviations: Chl – chlorophyll; *P*-rate – PS2 non-cyclic electron transport; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PS – photosystem; q_f – fast relaxing non-photochemical quenching; q_m – medium relaxing non-photochemical quenching; q_N – non-photochemical quenching; q_s – slow relaxing non-photochemical quenching; RC – reaction centre; RFp – rate of post-irradiation transient increase in Chl fluorescence.
Acknowledgements: This work was supported by the China National Natural Science Foundation (39870526) and the China State Key Basic Research and Development Plan (1999011700).

1996). Under high irradiance, a small pool of chloroplasts at the upper layer of leaf may be partially or fully photoinhibited. These chloroplasts form thus a photon absorbing shading shield protecting the majority of chloroplasts of the lower cell layers from photoinhibition and enabling normal or nearly normal photosynthetic CO_2 assimilation of the whole leaf. We speculated that if the irradiance response of leaf photosynthesis were determined by the non-invasive Chl fluorescence technique, maybe a single value of saturation irradiance could be obtained; this speculation was tested in young apple tree leaves.

Materials and methods

Plants: Two-year-old apple trees (*Malus pumila* Mill. cv. Tengmu No.1/*Malus hupehensis* Rehd) were grown in field. The tree stem with no branches reached a height of about 1.0 m. Three or four fully developed leaves of the medium part of tree stem were chosen for experiments.

Photosynthetic CO_2 assimilation and PS2 electron transport measurements: A gas exchange system *CIRAS-1* (*PP-Systems*, UK) was connected with a modulated fluorescence monitor system *FMS-2* (*Hansatech*, UK) for simultaneous photosynthetic CO_2 assimilation and PS2 electron transport measurements. The testing leaves were pre-irradiated under 400 μmol m^{-2} s^{-1} PPFD for 1.0 h and then were clamped into the *CIRAS-1* cuvette. The gas temperature (28±2 °C), relative humidity (75±5 %), CO_2 concentration (350±5 cm m^{-3}), and PPFD were controlled by the *CIRAS-1*. After pre-irradiation, leaves were irradiated with the required PPFD for 10 min in cuvette and P_N and Chl fluorescence parameters F_m', F_s, and F_0' were recorded by computers. The PS2 non-cyclic electron transport (*P*-rate) was calculated according to formula *P*-rate = $(F_m' - F_s)/F_m' \times$ PPFD (Genty *et al.* 1989). Chl fluorescence parameters F_0', F_s, and F_m' reflect irradiance-adapted fluorescence yield of leaf with PS2 RCs in open, partly closed, and fully closed states, respectively (Van Kooten and Snel 1990, Bilger and Schreiber 1990). We found that Chl fluorescence parameters $(F_s - F_0')$ and $(F_m' - F_0')$ represented the fluorescence yield of actually closed PS2 RCs caused by actinic irradiation and of the fully closed PS2 RCs caused by saturated pulse radiation, respectively. Thus $(F_s - F_0')/(F_m' - F_0')$ reflect the ratio of actinic radiation induced PS2 RC closure to the whole amount of active PS2 RCs.

Cyclic electron transport around PS1 and non-photochemical Chl fluorescence quenching components: Leaves were irradiated under the required PPFD for 10 min (actinic radiation was provided by *FMS-2*), then actinic radiation was switched off, and the rate of post-irradiation transient increase in Chl fluorescence (RFp) was measured according to Mi *et al.* (1995). During the 20-min dark adaptation switching off the actinic radiation, saturated pulse radiation was applied at 1 min alternation. The fast relaxing (q_f), medium relaxing (q_m), and slow relaxing (q_s) non-photochemical Chl fluorescence quenching were calculated according to Quick and Stitt (1989).

Chl fluorescence kinetics: Leaves were adapted in dark for 20 min after irradiation. A plant efficiency analyser (*PEA*, *Hansatech*, UK) was used to analyse Chl fluorescence kinetics. The PS2 maximal photochemical activity, ratio of the inactive PS2 RCs, and leaf potential ability for photosynthetic CO_2 assimilation were calculated according to formulae F_v/F_m, $(F_i - F_0)/(F_m - F_0)$, and F_d/F_s, respectively.

Results

With increase in PPFD, P_N of young apple tree leaves increased to saturation at PPFD of 600-1 100 μmol m^{-2} s^{-1}. P_N began to decrease above PPFD 1 100 μmol m^{-2} s^{-1} (Fig. 1*A*), while the PS2 non-cyclic electron transport (*P*-rate) had a distinct maximum at PPFD of 800 μmol m^{-2} s^{-1} (Fig. 1*B*). This phenomenon was explained by Schindler and Lichtenthaler (1996): when leaf is exposed to high irradiance, only the upper cell layer chloroplasts are photoinhibited. They then form a photon-absorbing shading shield to protect the majority of chloroplasts from photoinhibition. The latter chloroplasts then perform a normal photosynthetic CO_2 assimilation. Thus leaf P_N was nearly unchanged after saturation with photons. Hence P_N is not the actual irradiance response of leaf photosynthetic CO_2 assimilation at high irradiance. The upper cell layer chloroplasts in leaf upper side directly responded to PPFD, which was reflected in Chl fluorescence signals. Thus the PPFD of 800 μmol m^{-2} s^{-1} corresponded to the maximal *P*-rate, and this value was possibly the actual saturation irradiance of young apple tree leaf photosynthesis, while the decrease of *P*-rate from PPFD 900 μmol m^{-2} s^{-1} might reflect real photoinhibition.

After irradiation, leaves were darkened for 20 min. From the Chl fluorescence kinetics curves, the PS2 maximal photochemical activity F_v/F_m (Fig. 2*A*), the ratio of the PS2 inactive RCs $(F_i - F_0)/(F_m - F_0)$ (Fig. 2*B*), and the leaf potential photosynthetic ability of CO_2 assimilation F_d/F_s (Fig. 2*C*) were calculated. All these three parameters did not change under PPFD below 1 100 μmol m^{-2} s^{-1}, which was similar to P_N (Fig. 1*A*), while *P*-rate decreased incessantly from PPFD 900 μmol m^{-2} s^{-1} (Fig. 1*B*). Thus the *P*-rate decrease at PPFD of 800-1 100 μmol m^{-2} s^{-1}

was not induced by the loss of leaf potential photosynthetic activity. Chl fluorescence parameter F_v/F_m is commonly used as the criterion of photoinhibition (Adams *et al.* 1994). Here, F_v/F_m did not support the photoinhibition that we estimated using the *P*-rate.

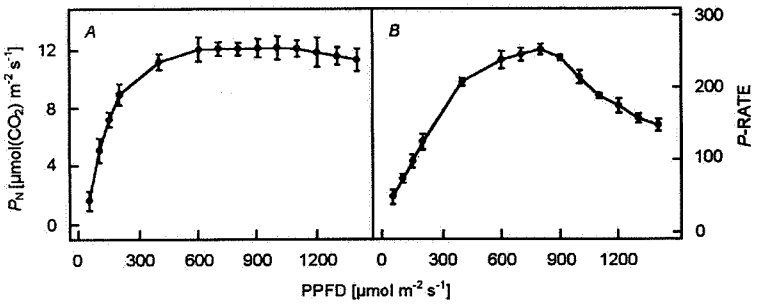

Fig. 1. Irradiance responses of net photosynthetic rate (P_N, *A*) and photosystem 2 electron transport (*P*-rate, *B*) in young apple tree leaves. P_N and *P*-rate were simultaneously measured under air temperature of 28±2 °C, relative humidity of 75±5 %, and CO_2 concentration of 350±5 cm^3 m^{-3}. Means (n = 4) with standard deviations.

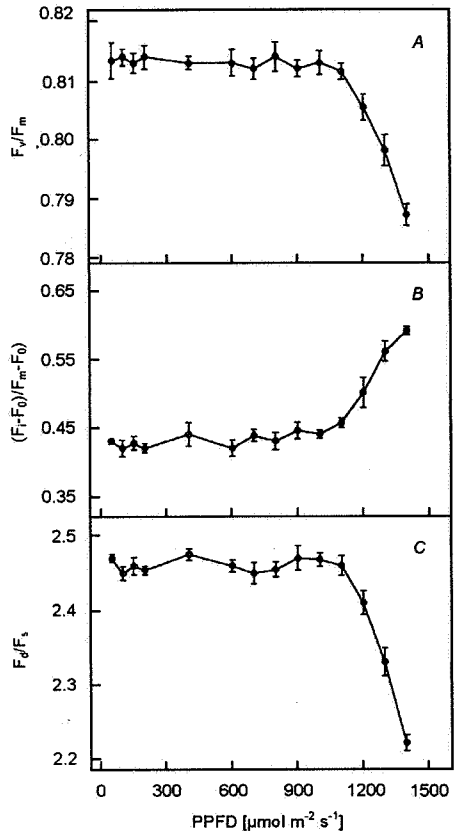

Fig. 2. Change of PS2 maximal photochemical activity (F_v/F_m, *A*), ratio of PS2 inactive reaction centres ($F_i - F_0$)/($F_m - F_0$) (*B*), and leaf potential ability for photosynthetic CO_2 assimilation (F_d/F_s, *C*) in young apple leaves after different irradiation. Measurements on leaves darkened for 20 min after irradiation. Means (n = 4) with standard deviations.

In normal photosynthesis, the photosynthetic photoreactions and dark reactions are coupled. NADPH and ATP are provided by photoreactions and the required $NADP^+$ and ADP are regenerated by dark reactions. If the supply of NADPH and ATP from photoreactions exceeds the demand of photosynthetic CO_2 assimilation, the excess NADPH and ATP could increase cyclic electron transport around PS1 through the reduction of plastoquinone pool (Mi *et al.* 1995). This may be reflected by the rate of transient post-irradiation increase in Chl fluorescence (RFp). PS2 photoreaction activity can be lowered by the reduction of plastoquinone pool. RFp and ($F_s - F_0'$)/($F_m' - F_0'$) of young apple tree leaves rapidly increased at PPFD above 800 μmol m^{-2} s^{-1} (Fig. 3*A,B*). Hence plenty of NADPH and ATP was produced which caused a decrease in PS2 photoreaction activity when the PPFD was higher than 800 μmol m^{-2} s^{-1}. Photoinhibition was originally defined by Kok as 'the debilitating effect of high intensities of visible light on the photosynthetic capability of green organism' (Kok 1956, Jones and Kok 1966). In agreement with this we found that photoinhibition occurred in young apple tree leaves at PPFD above 800 μmol m^{-2} s^{-1} as a response to irradiance.

To avoid potential damage of photosynthetic apparatus during photoinhibition, excess absorbed photons are dissipated as heat, which is reflected as an increase in energy-dependent non-photochemical Chl fluorescence quenching (q_N) (Krause and Weis 1991, Björkman and Demmig-Adams 1994). q_N is composed of several components: the fast relaxing component q_f= high energy quench, which is related to the formation of pH gradient; the medium relaxing component q_m = a quench related to state transitions of the photosystems; and the slow relaxing component q_s = photoinhibitory quench of PS2 (Demmig and Winter 1988). The three components can be determined by dark relaxation kinetics (Quick and Stitt 1989). Both q_f and q_m of young apple tree leaves showed a large enhancement at PPFD 800-1 000 μmol m^{-2} s^{-1} (Fig. 4*A,B*), while q_s increased distinctly from PPFD 1 200 μmol m^{-2} s^{-1} (Fig. 4*C*). These results indicated that the energy quenching mechanism and state transition mechanism co-operated to dissipate excess energy after saturation of photosynthesis at PPFD 800 μmol m^{-2} s^{-1}, and damage of the photosynthetic apparatus appeared from PPFD 1 200 μmol m^{-2} s^{-1}.

Discussion

Photosynthesis can be saturated at PPFD different for every plant. The irradiance dependence of P_N determined by gas exchange is commonly used to confirm saturation irradiance of the given plant type, but different values are mostly obtained (Prezelin 1981, Iqbal *et al.* 1997). Schindler and Lichtenthaler (1996) explain this fact by the photoinhibition in only a few upper layers of chloroplasts. These chloroplasts form a photon-absorbing shield protecting the rest of chloroplasts from photoinhibition. PS2 is the most sensitive component of photosynthetic apparatus (Aro *et al.* 1993, Long *et al.* 1994). Since the upper layer chloroplasts in leaf directly respond to radiant

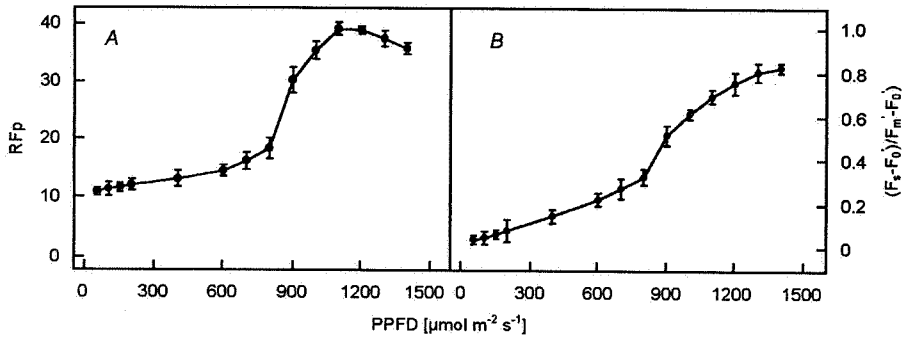

Fig. 3. Changes of the rate of post-irradiation transient increase in Chl fluorescence (RFp, *A*) and ratio of irradiation induced PS2 reaction centre closure $(F_s - F_0)/(F_m' - F_0')$ (*B*) in young apple leaves irradiated at different PPFD for 10 min. Means ($n = 4$) with standard deviations.

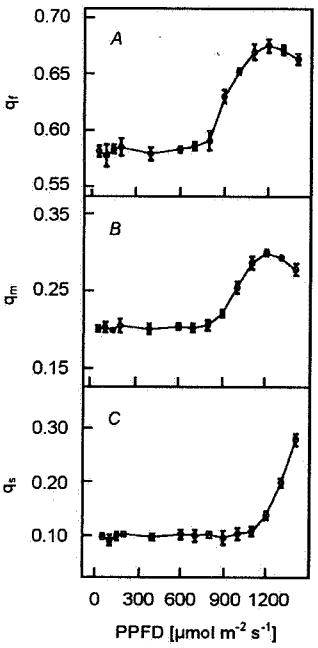

Fig. 4. Changes of the three chlorophyll fluorescence quenching components in young apple leaves irradiated by different PPFD: the fast phase (q_f, *A*), the medium phase (q_m, *B*), and the slow phase (q_s, *C*). Means ($n = 4$) with standard deviations.

energy and this is reflected in Chl fluorescence signals, the precise saturation irradiance of plant photosynthesis may be found using the Chl fluorescence technique. Using the saturation irradiance curves of PS2 non-cyclic electron transport, maximal *P*-rate was at the PPFD 800 μmol m^{-2} s^{-1} (Fig. 1*B*). This implies the maximum ability of young apple tree leaves to utilise the trapped photons through photosynthetic electron transport, but the *P*-rate decreased remarkably when PPFD exceeded 800 μmol m^{-2} s^{-1}. We propose that PPFD of 800 μmol m^{-2} s^{-1} is the saturation irradiance for photosynthesis of young apple tree leaves. But why *P*-rate was not maximal at PPFD of 600 μmol m^{-2} s^{-1} similarly to P_N (Fig. 1*A*)? This question can be explained by two assumptions: (*1*) P_N determination was largely affected also by factors other than irradiance, *i.e.* air temperature, vapour pressure deficit, CO_2 concentration, *etc.* (*2*) Electrons from photochemical reaction can be used in nitrate reduction and photorespiration, which are the metabolisms required for plant development. Mehler reaction can also consume electrons from the photochemical reaction. The enhancement of these metabolisms can improve photosynthetic electron transport in some extent after photosynthetic CO_2 assimilation is saturated and there are enough trapped photons in leaves.

In the absence of any stress factor, leaves acclimate to increasing PPFD by increasing their maximal photosynthetic capacity (Björkman 1981). However, no leaf can utilise all the absorbed photons for photosynthesis. Photoinhibition is a natural phenomenon that occurs at this occasion (Björkman and Demmig-Adams 1994). Up to present, there is no uniform definition of photoinhibition. Photoinhibition is generally manifested as a sustained decrease in the efficiency of photosynthetic energy conversion (*i.e.* a decrease in the rate of CO_2 uptake or O_2 evolution at limiting PPFD) or in the intrinsic efficiency of PS2 assessed using the ratio of variable to maximal yield of fluorescence (F_v/F_m) (Adams *et al.* 1994). Re-

cently, Chl fluorescence technique is the most common method used in photoinhibition research. In the irradiance response of photosynthesis of young apple tree leaves, Chl fluorescence parameters *P*-rate and F_v/F_m began to decrease at the PPFD of 900 μmol m^{-2} s^{-1} and 1 200 μmol m^{-2} s^{-1}, respectively (Figs. 1*B* and 2*A*). F_v/F_m was originally used to define photoinhibition *in vitro*, *e.g.* in chloroplasts and thylakoid membranes. Artificial damage of PS2 RCs was the most common result of this photoinhibition. Photoinhibition can also occur due to the enhancement of non-photochemical energy dissipation without any damage of the PS2 RCs (Long *et al.* 1994). We found that photoinhibition in young apple tree leaves occurred first at PPFD of 900 μmol m^{-2} s^{-1}. When PPFD increased above 900 μmol m^{-2} s^{-1}, young apple tree leaf RFp and $(F_s - F_0')/(F_m' - Fo')$ increased distinctly (Fig. 3*A,B*), but F_d/F_s was nearly unchanged (Fig. 2*C*). This result indicates that the supply of NADPH and ATP exceeded the demand of photosynthetic CO_2 assimilation. The excess of NADPH and ATP accelerated cyclic electron transport around PS1 and then caused much more reduced PS2 RCs in light, which induced a decrease in *P*-rate and photoinhibition. Change of q_s pronounced the photoinhibitory damage in young apple tree leaves at PPFD of 1 200 μmol m^{-2} s^{-1} (Fig. 4*C*). In this kind of photoinhibition, leaves required repair mechanisms for the photosynthetic activity recovery, *e.g.* synthesis and integration of the D1 protein (Quick and Stitt 1989), which was also supported by the F_v/F_m change (Fig. 2*A*). In the photoinhibition at PPFD 900-1 100 μmol m^{-2} s^{-1}, only the value of two non-photochemical Chl fluorescence-quenching components, q_f and q_m, increased (Fig. 4*A,B*). q_s nearly did not change (Fig. 4*C*), which indicated that the excess photons hardly did harm to PS2 RCs, but were mainly dissipated by the energy quenching and state transition mechanisms. The energy quenching and state transition mechanisms can quickly recover in dark, their $t_{1/2}<1$ min and $t_{1/2}<10$ min for q_f and q_m, respectively (Horton and Hague 1988). Therefore leaves can still perform the original F_v/F_m in the non-photochemical energy dissipation induced photoinhibition after short dark adaptation. Thus F_v/F_m is not the right index for defining photoinhibition without PS2 RCs damage and we propose to use the Chl fluorescence parameter *P*-rate for correct definition of photoinhibition.

References

Adams, W.W., Demmig-Adams, B., Verhoeven, A.S., Barker, D.H.: 'Photoinhibition' during winter stress: involvement of sustained xanthophylls cycle-dependent energy dissipation. – Aust. J. Plant Physiol. **22**: 261-276, 1994.

Aro, E.M., Virgin, I., Anderson, B.: Photoinhibition of photosystem II: Inactivation, protein damage and turnover. – Biochim. biophys. Acta **1143**: 113-134, 1993.

Bilger, W., Schreiber, U.: Chlorophyll fluorescence as an indicator of stress-induced damage to the photosynthetic apparatus: Effects of heat-stress in isolated chloroplasts. – Photosynth. Res. **25**: 161-171, 1990.

Björkman, O.: Responses to different quantum flux densities. – In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.): Physiological Plant Ecology. I. Pp. 57-107. Springer-Verlag, Berlin – Heidelberg – New York 1981.

Björkman, O., Demmig-Adams, B.: Regulation of photosynthetic light energy capture, conversion and dissipation in leaves of higher plant. – In: Schulze, E.D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 17-47. Springer-Verlag, Berlin 1994.

Demmig, B., Winter, K.: Characterisation of three components of non-photochemical fluorescence quenching and their response to photoinhibition. – Aust. J. Plant Physiol. **15**: 163-177, 1988.

Falkowski, P.G., Greene, R., Kolber, Z.: Light utilization and photoinhibition of photosynthesis in marine phytoplankton. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field. Pp. 407-432. BIOS, Oxford 1994.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Horton, P., Hague, A.: Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non-photochemical quenching. – Biochim. biophys. Acta **932**: 107-115, 1988.

Iqbal, R.M., Rao, A.R., Rasul, E., Wahid, A.: Mathematical Models and Response Functions in Photosynthesis. – Pp. 803-810. Marcel Dekker, New York 1997.

Jones, L.W., Kok, B.: Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. – Plant Physiol. **41**: 1037-1043, 1966.

Kok, B.: On the inhibition of photosynthesis by intense light. – Biochim. biophys. Acta **21**: 234-244, 1956.

Krall, J.P., Edwards, G.E.: Relationship between photosystem II activity and CO_2 fixation in leaves. – Physiol. Plant. **86**: 180-187, 1992.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. – Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 633-662, 1994.

Mi, H., Endo, T., Ogawa, T., Asada, K.: Thylakoid membrane-bond, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic election transport in the cyanobacterium *Synechocystis* sp. PCC6803. – Plant Cell Physiol. **36**: 661-668, 1995.

Powles, S.B., Osmond, C.B., Thorne, S.W.: Photoinhibition of intact attached leaves of C_3 plants illuminated in the absence of both carbon dioxide and of photorespiration. – Plant Physiol. **64**: 982-988, 1979.

Prezelin, B.B.: Light reactions in photosynthesis. – Can. Bull. Fish. aquat. Sci. **210**: 1-43, 1981.

Quick, W.P., Stitt, M.: An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. – Biochim. biophys. Acta **997**: 287-296, 1989.

Schindler, C., Lichtenthaler, H.K.: Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the course of a sunny and a cloudy day. – J. Plant Physiol. **148**: 399-412, 1996.

Van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.