PHOTOSYNTHETICA 36 (1-2): 61-67, 1999

Diurnal fluctuations in photochemical activities of chloroplasts in field grown *Cyamopsis tetragonoloba* seedlings

K. LINGAKUMAR and G. KULANDAIVELU*

School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

Abstract

In field-grown *Cyamopsis* seedlings, distinct changes were found in the rates of photosystems (PS) 2 and 1 activities at different time of the day. Maximum PS2 activity was at around 11:00 h and decreased thereafter. On the contrary, PS1 activity continued to increase up to 14:00 h and declined in evening hours. Significant energy transfer from PS2 to PS1 was evident during the morning and evening hours of the day whereas a slow excitation of PS2 and energy transfer was favoured during noon hours.

Additional key words: chlorophyll fluorescence; photochemical activities; photosystems 1 and 2.

Introduction

Radiant energy is essential not only as the driving force of photosynthesis but also as a trigger and modulator of photomorphogenic responses. The daily quantum flux available varies seasonally, diurnally, and spatially. Plants are able to adapt to varying irradiance which results from genetic adaptation (Boardman 1977). The short term regulations such as state transitions involve phosphorylation and dephosphorylation of the light-harvesting complex 2 (LHC2) and other complexes that in turn affect the mobility of antenna complexes and their association with PS1 and PS2 (Barber 1982, Bennett 1991, Allen 1992). The genetically programmed regulation is correlated to the oscillation in the amount of LHC2 in thylakoid membranes of wheat (Busheva *et al.* 1991) and pea (Adamska *et al.* 1991) leaves. Among the LHC proteins, the 25 kDa protein phosphorylates rapidly. Its preferential role in diurnal adjustment of the size of LHC was also reported (Larsson and

Received 14 August 1997, *accepted* 6 August 1998.
*Author for correspondence; fax: +91 452 858485; e-mail: gkplant@pronet.xlweb.com
Abbreviations: BV - benzylviologen; Chl - chlorophyll; $DCPIPH_2$ - reduced dichlorophenol indophenol; LHC2 - light-harvesting chlorophyll of PS2; MV - methylviologen; PS - photosystem; PPFD - photosynthetic photon flux density.
Acknowledgement: We thank the Council of Scientific and Industrial Research for the Senior Research Associateship to one of us (K.L.).

Andersson 1985, Liker and Garab 1995). A wide variety of plant species dissipate the excess absorbed radiation *via* xanthophyll cycle-dependent energy dissipation (Demmig-Adams and Adams 1996). Our experiment was designed to detect the diurnal fluctuations in the functioning of chloroplasts in terms of photochemical activities, fluorescence kinetics, and low temperature fluorescence emission.

Materials and methods

Seedlings of *Cyamopsis tetragonoloba* L. grown in pots filled with garden soil were exposed to solar radiation. The incidental PPFDs were 450 (at 07:00 h), 4 200 (12:00 h) and 320 μmol m^{-2} s^{-1} (17:00 h). Five-d-old seedlings were analyzed for diurnal fluctuations in the functioning and composition of photosystems. Type II broken chloroplasts were isolated from the cotyledonary leaves for photosynthetic measurements according to Reeves and Hall (1973). Chlorophyll (Chl) content was estimated according to the method of Wellburn and Lichtenthaler (1984). The photochemical electron transport activities were performed as described in Lingakumar and Kulandaivelu (1996). Chl *a* fluorescence induction kinetics and 77 K fluorescence emission spectra were followed in intact leaves and isolated chloroplasts, respectively, according to Kulandaivelu and Daniell (1980) and Lingakumar and Kulandaivelu (1993).

Results and discussion

Changes in the overall rate ($H_2O \rightarrow MV$), PS2 ($H_2O \rightarrow BQ$), and PS1 ($DCPIPH_2 \rightarrow MV$) activities followed in *Cyamopsis* chloroplasts are shown in Fig. 1. The PS2 activity was high in the early hours of day and declined in the afternoon hours. On the contrary, PS1-mediated electron transport showed linear increase up to 14:00 h and a drastic decrease thereafter. Similar changes were also observed for the whole-chain electron transport. These photochemical changes resemble those of seedlings exposed to different irradiances (Kulandaivelu and Sampath 1983). The decrease in photosynthetic capacity measured in terms of PS2 and PS1 activity during the late hours of the day is attributed to the alterations in composition of both the Chl proteins and electron transport complexes (Leong and Anderson 1983). The marked decrease in PS2 activity during the midnoon hours indicates the regulation of photosystems to avoid high irradiance. Such a low PS2 efficiency during the noon hours, especially under high sunlight, could be due to high energy dissipation in the PS2 antennae (Demmig-Adams and Adams 1992). High energy dissipation was detected by monitoring the efficiencies of conversion from violaxanthin to antheraxanthin/zeaxanthin (Demmig-Adams and Adams 1996). Such regulation was further confirmed by the Chl *a* fluorescence induction curves and 77 K fluorescence emission spectra from intact leaves and isolated chloroplasts.

Changes in the kinetics of Chl *a* fluorescence are a good probe in tracing the primary photosynthetic events and also in monitoring the plant response to any

Table 1. Relative values of F686, F695, F730, F730/F686, half-rise time ($t_{1/2}$) of the Chl fluorescence, and ratio of variable to maximum fluorescence (F_v/F_m) in chloroplasts isolated from *Cyamopsis* seedlings collected at different time of the day. Measurements were made soon after the isolation of chloroplasts. Values were obtained from the emission spectra followed at 77 K after excitation with 430 nm. For half-rise and F_v/F_m means ±SE are given, $n = 5$.

Time of the day [h]	F_{686}	F_{695}	F_{730}	F_{730}/F_{686}	$t_{1/2}$	F_v/F_m
07:00	28	26	20	0.714	200±25	0.77±0.02
12:00	40	27	33	0.825	210±32	0.63±0.04
19:00	32	30	40	1.250	260±42	0.57±0.03

environmental stress. Fast and slow fluorescence transients recorded with leaves collected from seedlings at different time of the day are shown in Fig. 2. The morning samples showed a fast O-P rise while the noon and evening samples had a gradual rise. A change in the F_0 level was noticed in these samples. A progressive decline in F_v/F_m ratio was found from morning to evening (Table 1). Samples

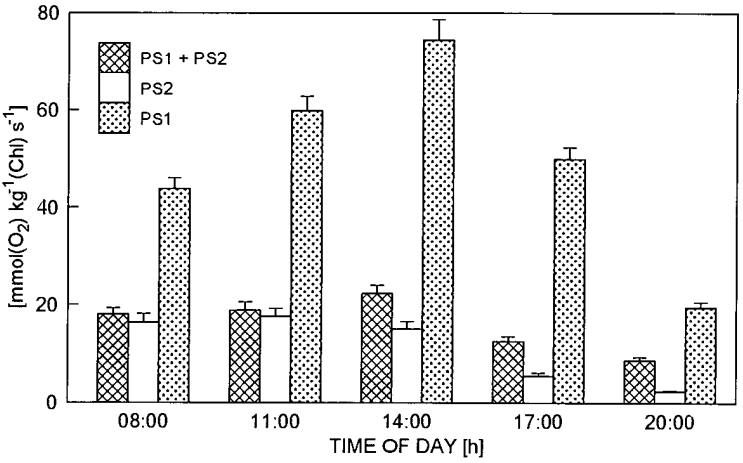

Fig. 1. Changes in photochemical activities of *Cyamopsis* chloroplasts at different time of the day. Whole chain ($H_2O \rightarrow MV$), PS2 ($H_2O \rightarrow BQ$), and PS1 ($DCPIPH_2 \rightarrow MV$) activities were assayed using a *Hansatech* oxygen electrode. The assay buffer consisted of 100 mM sucrose, 20 mM Tris-HCl, pH 7.5, 5 mM NaCl, 5 mM $MgCl_2$. The final concentrations of the artificial electron acceptors and donors were 100 μM BQ, 100 μM MV, 50 μM DCPIP, 2 mM sodium ascorbate, and 5 μM NaN_3. The chloroplasts containing 20 μg Chl were used for all the measurements. Actinic irradiance at the sample surface of the electrode was 150 W m^{-2}. Each value is an average of 5 measurements.

collected at 12:00 and 19:00 h showed a 18 and 26 % reduction in F_v/F_m ratio when compared to the 07:00 h sample. The $t_{1/2}$ (half rise time of F_0-F_p) was shorter in the morning, and 5 and 30 % increases were found in the noon and evening hours, respectively. In contrast to fast changes occurring in Chl fluorescence, prolonged irradiation of dark-adapted leaves leads to gradual activation of Calvin cycle enzymes (Karapetyan and Bukhov 1986). Thus the slow transients of Chl fluorescence exhibit

an interplay between overall electron transport and carbon reduction cycle (Ögren 1990). The most activated enzyme under light is the terminal enzyme of the electron transport chain, the Fd^{2+}-NADP reductase, and that leads to the decline of fluorescence level to S (the P-S decline) (Satoh 1981).

A sharp P-S decline was noticed only in the noon sample, and a prominent secondary rise to M was evident in the morning and evening samples. The SMT sector of the slow fluorescence is associated with the redistribution of excitation energy of absorbed quanta between PS2 and PS1 and particularly in favour of the latter (Chow *et al.* 1981). Hence, during the noon hours the chloroplast machinery switches off the PS2 activity at least temporarily. Gradual reduction in PS2 activity during late hours of the day was not so evident in the fluorescence induction curves and was not the same as manifested in the photochemical activities. This is because the F_v/F_m values recover rapidly in the dark (Cornic *et al.* 1992). The duration of dark incubation, especially in the non-modulated fluorescence measurements, is too long (Kyparissis *et al.* 1995) to affect the F_v/F_m values.

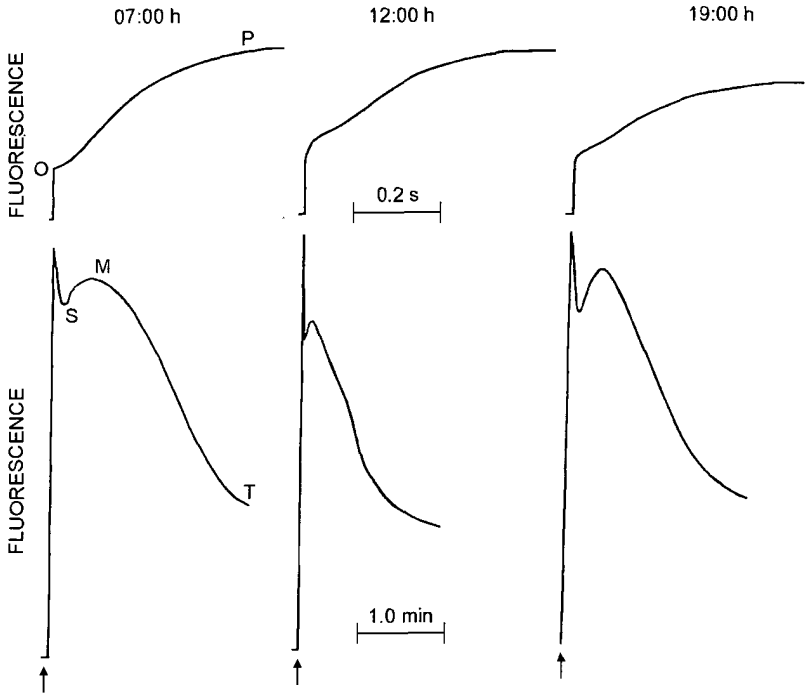

Fig. 2. Typical fluorescence transients (fast and slow) in intact *Cyamopsis* leaves collected at different time of the day. All leaf samples were incubated in the dark for 30 min prior to fluorescence measurements. The abscissae indicate the switching on/off of the excitation (blue) radiation.

At 77 K, characteristic emission by Chl-protein complexes such as PS1, PS2, and LHCP can be distinguished (Fig. 3). When the chloroplasts were excited at 430 nm, prominent bands were observed at 686, 695, and 730 nm: these bands are attributed

to emission by LHC and antenna pigments of PS1 and PS2, respectively (Bose 1982). The F_{695} peak was prominent at 07:00 and 19:00 h. Such prominence indicates that the PS2 core complex is probably reduced in size due to high ambient irradiation

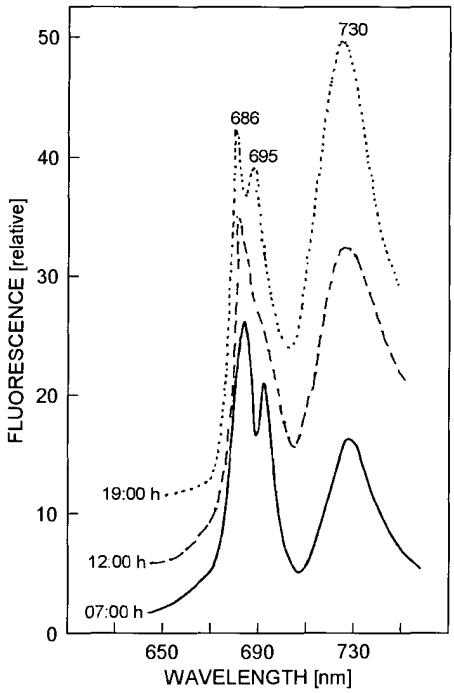

Fig. 3. Low temperature (77 K) fluorescence emission spectra of the chloroplasts isolated from *Cyamopsis* seedlings at different time of the day. Chloroplasts were isolated from leaves collected from 3-d-old seedlings. Chloroplasts were suspended at a final Chl concentration of 5 g m^{-3} in reaction buffer containing 70 % (v/v) glycerol. The chloroplasts were excited at 430 nm.

in the noon hours or less energy transfer to the photosynthetic reaction centres. In contrast to this, the F_{730} arises from a special pigment absorbing at 705 nm that acts as an energy trap of PS1 antenna (Butler 1961, Satoh and Butler 1978). Thus the diminution of the 730 nm peak in the 07:00 h sample may be due to slow activation of the PS1 antenna. Moreover, the long-wavelength absorbing pigments of PS1 receive energy from the closely associated LHCP (Thornber *et al.* 1979). Thus, the 77 K fluorescence measurements show that at noon the overexcitation of PS2 is avoided through a concomitant increased energy transfer towards PS1. As the 70 K fluorescence spectra (Table 1) show, the F_{730}/F_{686} ratio was fairly high in the noon and evening samples. Since the LHC2 is the predominant integral protein complex of thylakoid membranes in green algae and higher plants, these antenna complexes have a regulatory role in short-term and long-term processes of adaptation to irradiance apart from the light-harvesting function (Anderson and Andersson 1988). The LHC2 gene expression is controlled by a circadian cycle (Piechulla and Gruissem 1987, Nagy *et al.* 1987, Piechulla 1993). Liker and Garab (1995) proved that the 27 kDa of LHC2 is more fluctuating in term of its size during the day hours of high solar irradiances where a preferential increase in PS1 activity is favoured by lowering the activity of PS2. Such control may also exist for the xanthophyll cycle and its intermediates. Thus the present investigation confirms that during summer periods

with high solar fluxes, the PS2 activity is maximised during the forenoon hours, but later the PS1 activity is more fast.

References

Adamska, I., Scheel, B., Kloppstech, K.: Circadian oscillation of nuclear-encoded chloroplast proteins in pea (*Pisum sativum*). - Plant mol. Biol. **17**: 1055-1065, 1991.

Allen, J.F.: Protein phosphorylation in regulation of photosynthesis. - Biochim. biophys. Acta **1098**: 275-335, 1992.

Anderson, J.M., Andersson, B.: The dynamic photosynthetic membrane and regulation of solar energy conversion. - Trends biochem. Sci. **13**: 351-355, 1988.

Barber, J.: Influence of surface charges on thylakoid structure and function. - Annu. Rev. Plant Physiol. **33**: 261-295, 1982.

Bennett, J.: Protein phosphorylation in green plant chloroplasts. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 281-311, 1991.

Boardman, N.K.: Comparative photosynthesis of sun and shade plants. - Annu. Rev. Plant Physiol. **28**: 355-377, 1977.

Bose, S.: Chlorophyll fluorescence in green plants and energy transfer pathways in photosynthesis. - Photochem. Photobiol. **36**: 725-731, 1982.

Busheva, M., Garab, G., Liker, E., Tóth, Z., Széll, M., Nagy, F.: Diurnal fluctuations in the content and functional properties of the light harvesting chlorophyll *a/b* complex in thylakoid membranes. Correlation with the diurnal rhythm of the mRNA level. - Plant Physiol. **95**: 997-1003, 1991.

Butler, W.L.: A far-red absorbing form of chlorophyll *in vivo*. - Arch. Biochem. Biophys. **93**: 413-422, 1961.

Chow, W.S., Telfer, A., Chapman, D.J., Barber, J.: State 1–state 2 transitions in leaves and its association with ATP-induced chlorophyll fluorescence quenching. - Biochim. biophys. Acta **638**: 60-68, 1981.

Cornic, G., Ghashghaie, J., Genty, B., Briantais, J.-M.: Leaf photosynthesis is resistant to a mild drought stress. - Photosynthetica **27**: 295-309, 1992.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Demmig-Adams, B., Adams, W.W., III: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. - Trends Plant Sci. **1**: 21-26, 1996.

Karapetyan, N.V., Bukhov, N.G.: [Variable chlorophyll fluorescence as an index of the physiological state of plants.] - Fiziol. Rast. **33**: 1013-1026, 1986. [In Russ.]

Kulandaivelu, G., Daniell, H.: Dichlorophenyl dimethylurea (DCMU) induced increase in chlorophyll *a* fluorescence intensity - an index of photosynthetic oxygen evolution in leaves, chloroplasts and algae. - Physiol. Plant. **48**: 385-388, 1980.

Kulandaivelu, G., Sampath, P.: Changes in photosynthetic apparatus upon shading of plants. - Plant Physiol. Biochem. **10**: 151-160, 1983.

Kyparissis, A., Petropoulou, Y., Manetas, Y.: Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., *Labiatae*) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. - J. exp. Bot. **46**: 1825-1832, 1995.

Larsson, U.K., Andersson, B.: Different degrees of phosphorylation and lateral mobility of two polypeptides belonging to the light harvesting complex of Photosystem II. - Biochim. biophys. Acta **809**: 396-402, 1985.

Leong, T.-Y., Anderson, J.M.: Changes in composition and function of thylakoid membranes as a result of photosynthetic adaptation of chloroplasts from pea plants grown under different light conditions. - Biochim. biophys. Acta **723**: 391-399, 1983.

Liker, E., Garab, G.: Diurnal fluctuation in the composition of chlorophyll *a/b* light harvesting antenna of photosystem II in young wheat leaves. - Physiol. Plant. **93**: 187-190, 1995.

Lingakumar, K., Kulandaivelu, G.: Changes induced by ultraviolet-B radiation in vegetative growth, foliar characteristics and photosynthetic activities in *Vigna unguiculata*. - Aust. J. Plant Physiol. **20**: 299-308, 1993.

Lingakumar, K., Kulandaivelu, G.: Reversal of ultraviolet-B (280-320 nm) radiation induced changes in *Vigna unguiculata* L. chloroplasts by supplemental "white light". - Photosynthetica **32**: 463-469, 1996.

Nagy, F., Kay, S.A., Chua, N.H.: The analysis of gene expression in transgenic plants. - In: Gelvin, S.B., Schilperoot, R.A. (ed.): Plant Gene Research Manual. Pp. 1-29. Kluwer Academic Press, Dordrecht 1987.

Ögren, E.: Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. - Plant Physiol. **93**: 1280-1285, 1990.

Piechulla, B.: 'Circadian clock' directs the expression of plant genes. - Plant mol. Biol. **22**: 533-542, 1993.

Piechulla, B., Gruissem, W.: Diurnal mRNA fluctuations of nuclear and plastid genes in developing tomato fruits. - EMBO J. **6**: 3593-3599, 1987.

Reeves, S.G., Hall, D.O.: The stoichiometry (ATP/$2e^-$ ratio) of non-cyclic photophosphorylation in isolated spinach chloroplasts. - Biochim. biophys. Acta **314**: 66-78, 1973.

Satoh, K.: Fluorescence induction and activity of ferredoxin-$NADP^+$ reductase in *Bryopsis* chloroplasts. - Biochim. biophys. Acta **638**: 327-333, 1981.

Satoh, K., Butler, W.L.: Low temperature spectral properties of subchloroplast fractions purified from spinach. - Plant Physiol. **61**: 373-379, 1978.

Thornber, J.P., Markwell, J.P., Reinman, S.: Plant chlorophyll-protein complexes: recent advances. - Photochem. Photobiol. **29**: 1205-1216, 1979.

Wellburn, A.R., Lichtenthaler, H.: Formulae and program to determine total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. - In: Sybesma, C. (ed.): Advances in Photosynthesis Research. Vol. II. Pp. 9-12. Martinus Nijhoff/Dr W. Junk Publ., The Hague - Boston - Lancaster 1984.