Changes in the Stoichiometry of Photosystem II Components as an Adaptive Response to High-Light and Low-Light Conditions during Growth

Aloysius Wild, Matthias Höpfner, Wolfgang Rühle, and Michael Richter

Institut für Allgemeine Botanik der Universität, Saarstraße 21, D-6500 Mainz, Bundesrepublik Deutschland

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The effect of different growth light intensities (60 $W \cdot m^{-2}$, 6 $W \cdot m^{-2}$) on the performance of the photosynthetic apparatus of mustard plants (*Sinapis alba* L.) was studied. A distinct decrease in photosystem II content per chlorophyll under low-light conditions compared to high-light conditions was found. For P-680 as well as for Q_A and Q_B protein the molar ratio between high-light and low-light plants was 1.4 whereas the respective concentrations per chlorophyll showed some variations for P-680 and Q_A on the one and Q_B protein on the other hand.

In addition to the study of photosystem II components, the concentrations of PQ, Cyt *f*, and P-700 were measured. The light regime during growth had no effect on the amount of P-700 per chlorophyll but there were large differences with respect to PQ and Cyt *f*. The molar ratio for Cyt *f* and PQ between high- and low-light leaves was 2.2 and 1.9, respectively.

Two models are proposed, showing the functional organization of the pigment system and the electron transport chain in thylakoids of high-light and low-light leaves of mustard plants.

Introduction

Among the adaptive responses of plants to different ecological conditions the adaptation to different light intensities is one of the most important ones. Light intensity and light quality have a considerable influence as well on the morphology and anatomy of plants as on photosynthesis and other primary and secondary metabolic processes. The effects of growth light conditions on the photosynthetic rate and on the photosynthetic apparatus have been studied intensely in the last few years. Considerable progress has been made in the clarification of these adaptive processes [1–4].

Concerning the photosynthetic electron transport system, a considerable decrease in the concentration of most of the redox components under LL conditions could be shown while growth light regime has at best a small effect on the content of P-700 trapping centers. There were no marked differences between sun and shade species in this respect [4–6].

Since PS II content was also found not to vary remarkably [1], the PS II/PS I stoichiometry was regarded to remain approximately constant during HL-LL-adaptation [2, 4]. Later on, hints on seasonal changes in photosystem stoichiometry were obtained [7]. Björkman [6] then postulated an increased P-680 content in shade adapted chloroplasts so that shade leaves should have a higher PS II/PS I ratio than sun leaves.

With regard to light intensity, however, this conception was contradicted by investigations of Sampath and Kulandaivelu [8] and Leong and Anderson [9], who found less PS II per Chl and thus a lower PS II/PS I ratio in the LL adapted plants.

On the other hand, the effects of light quality seem to be different from those of light intensity and they are obviously in agreement with Björkman's proposal [10–12].

Because in most studies the PS II content was determined by measuring only one single component of the PS II complex, we checked the three components P-680, Q_A, and Q_B together in the same plant material. Thus, a direct comparison of the adaptive behaviour of these PS II redox carriers was made possible. For the measurement of P-680 we additionally used two different methods.

Abbreviations: Chl, chlorophyll; Cyt *f*, cytochrome *f*; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HL, high light; LHCP, light-harvesting chlorophyll *a*/*b*-protein; LL, low light; PAGE, polyacrylamide gel electrophoresis; PQ, plastoquinone pool; PS, photosystem; P-680, reaction center of PS II; P-700, reaction center of PS I; Q_A, primary quinone electron acceptor of PS II; Q_B, secondary quinone electron acceptor protein; SDS, sodium dodecyl sulphate; Tris, tris (hydroxy-methyl)-aminomethane.

Reprint requests to Prof. Dr. A. Wild.

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Materials and Methods

Plant material

Mustard plants (*Sinapis alba* L.) were grown as reported previously [13]. Primary and secondary leaves were taken from 14 day old (HL: 60 $W \cdot m^{-2}$) or 26 day old (LL: 6 $W \cdot m^{-2}$) plants to ensure that they were in a comparable developmental state (see [4]).

Class C chloroplasts were isolated as described earlier [13]. The Chl content was determined according to Ziegler and Egle [14].

Measurement of P-680

The determination of the P-680 content by flash-light experiments (Combi Spark System, Impulsphysik Ltd, Hamburg) was based on the classical investigations of Emerson and Arnold [15]. Saturating repetitive argon-flashes of 0.5 μs half-time were used at a frequency of 5 Hz. O_2 and H^+ release were measured at 20 °C in a ferricyanide (1 $mmol \cdot l^{-1}$) mediated Hill-reaction using a Clark-type O_2-electrode (YSI Kettering) and a glass-pH-electrode (type N 62, Schott Ltd, Mainz), respectively.

Q_A and PQ

Chl *a* fluorescence was measured using a bifurcated fiber optics system as described by Schreiber [16]. Excitation was by blue light of 450 nm (Schott BG 28 filter, 2 mm), irradiance was 5 $W \cdot m^{-2}$. The S-20 type photomultiplier was protected against actinic light by a 3 mm RG 665 filter (Schott). The time-course of fluorescence emission was stored digitally (Vuko VK 12-12 transient recorder) and brought to the same maximum fluorescence level by means of a computer (CBM 4032, Commodore). Q_A was calculated from the area above the fluorescence induction curve (ΔF) in the presence of DCMU (1 $mmol \cdot l^{-1}$). The approximate size of the PQ pool was determined from the ΔF received without addition of an electron transport inhibitor; the addition of DBMIB to the thylakoid suspension resulted only in an insignificant reduction of ΔF. In order to calculate absolute concentrations of Q_A and PQ from the respective ΔF, which was given as relative units, we added defined concentrations of the artificial electron acceptor ferricyanide (0–45 $\mu mol \cdot l^{-1}$) to the thylakoid suspension, thus inducing proportional changes of ΔF as shown in Fig. 1 (see [17]). We found different ratios between ΔF and the size of the respective ferricyanide pool for HL and LL thylakoids.

Q_B protein

Measurements were performed according to the method of Tischer and Strotmann [18], except that binding of the labelled inhibitor was carried out at 0 °C and that the final alcohol concentration in the reaction medium was only 0.5%. The final Chl concentration was about 50 $\mu g \cdot ml^{-1}$. [^{14}C]atrazine (25 Ci/mol) was purchased from Amersham. The concentration of specific binding sites was calculated from the double reciprocal plots (mol $Chl \cdot mol^{-1}$ bound inhibitor *vs* μM^{-1} free inhibitor; see Fig. 2). Radioactivity was measured using a scintillation counter BF 8000 Multi User LSC (Berthold).

P-700 content was estimated according to Hiyama and Ke [19] from the ferricyanide oxidized (60 $mmol \cdot l^{-1}$) minus sodium ascorbate reduced (240 $mmol \cdot l^{-1}$) spectrum.

Cyt *f* concentration was determined from the ferricyanide oxidized (75 $mmol \cdot l^{-1}$) minus hydroquinone reduced (75 $mmol \cdot l^{-1}$) spectrum as described by Bendall *et al.* [20]. In both cases the spectrum was recorded by a Shimadzu spectrophotometer MPS 2000.

Separation of Chl protein complexes by SDS-PAGE using a Tris-borate buffer and determination of the Chl content of the gel band was performed according to Wild *et al.* [21].

Results

P-680 determinations are based on saturating flash-light intensities as it is shown here by the light-dependence curves of the proton release (Fig. 3). All the determinations were carried out by 100% flash intensity.

Using the Lineweaver-Burk plot, an extrapolation to unlimited flash intensity could be done (data not shown). These results did not differ significantly from those obtained directly by full flash-light intensity (100% in Fig. 3). In the O_2 as well as pH measurements, a distinct reduction of P-680 content in LL thylakoids could be established compared to the respective values of HL membranes. A summary of all results is given in Table I.

Table I. Content of redox components (molecules per 1000 molecules Chl) and the respective HL/LL ratio of *Sinapis alba* plants grown under HL ($60\ W \cdot m^{-2}$) and LL ($6\ W \cdot m^{-2}$) conditions. P-680 was determined by O_2 as well as by H^+ release. The PS II values are the mean of P-680 and Q_A measurements. Each value is the mean of 9–15 determinations.

Component	Molecules/1000 molecules Chl		HL/LL
	HL	LL	
P-680 O_2	2.3 ± 0.3	1.6 ± 0.2	1.4
H^+	2.4 ± 0.3	1.7 ± 0.2	1.4
Q_A	2.7 ± 0.5	1.9 ± 0.3	1.4
PS II	2.5 ± 0.3	1.7 ± 0.2	1.4
Q_B protein	3.4 ± 0.1	2.4 ± 0.1	1.4
P-700	2.5 ± 0.1	2.5 ± 0.1	1.0
Cyt *f*	2.1 ± 0.1	0.9 ± 0.1	2.2
PQ	25 ± 5	13 ± 2	1.9

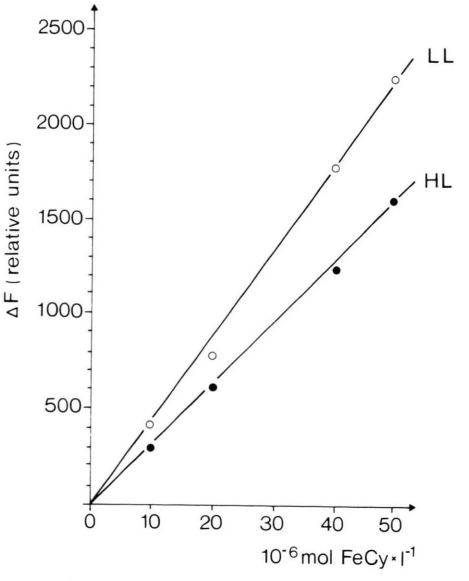

Fig. 1. The relation between the FeCy induced increase of the area above the fluorescence induction curve (ΔF) and the concentration of the electron acceptor ferricyanide (FeCy) added to HL (●) and LL (○) thylakoids.

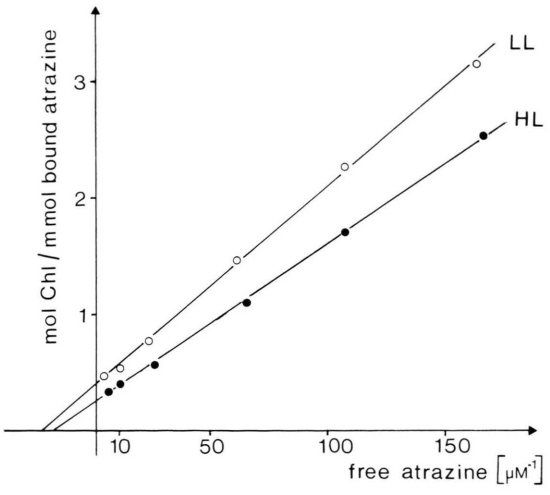

Fig. 2. Double-reciprocal-plot of binding of [^{14}C]atrazine by HL (●) and LL (○) thylakoids of *Sinapis alba*. The total concentrations of specific binding sites are obtained from the respective intercepts with the *y*-axis.

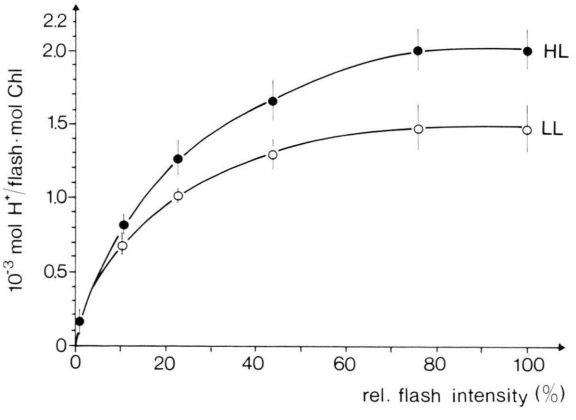

Fig. 3. Light dependence curves of H^+ release during exposure of HL (●) and LL (○) thylakoids to repetitive short flashes (flash yield is plotted versus relative flash intensity). Chl concentration of the respective thylakoid suspension was 3 $\mu g \cdot ml^{-1}$.

In the run of the calculation of Q_A content, no significant differences in ΔF could be found after DCMU treatment of HL and LL thylacoids, respectively (see [1]). Nevertheless we established a decrease in Q_A content under LL conditions, that paralleled exactly the P-680 concentration change. This phenomenon was the result of differences in electron transport activity between HL and LL thylakoids, leading to different ratios between ΔF and the size of the electron acceptor pool (Fig. 1). It is not yet clear what these differences in electron transport activity under rate limiting light conditions mean. There are

indications that the quantum yield of the ferricyanide mediated Hill-reaction is lower in thylakoids of LL chloroplasts than in those of HL chloroplasts [22].

The identical values of the HL/LL ratio of Q_A, Q_B protein, and of P-680 (Table I) indicate a fixed stoichiometry between these PS II components. To evaluate these data, it has to be considered that systematical errors concerning the different determination methods affect the experimental results for HL and LL material to the same extent. Thus, the HL/LL ratio should contrary to the respective concentrations of the redox-components not be influenced by such inherent methodical problems.

With regard to the P-680 and Q_A determinations which are based on an active photosynthetic reaction, inactivation processes during chloroplast isolation would lead to a reduced electron transport activity. Thus, because of the diminished O_2- and H^+-evolution, too little P-680 per Chl would be detected while on the other hand the increased area ΔF above the fluorescence curve would lead to an overestimation of the Q_A content per Chl. Nevertheless, no significant differences between the determined P-680 and Q_A concentrations where detectable.

For Q_B protein estimation a binding assay is used and no full electron transport activity is required. In the applied range of [^{14}C]atrazine concentration ($0.4-0.02$ μmol·l^{-1}) unspecific binding reactions and thus an overestimation of Q_B protein concentration can be excluded (see [18]). Because inhibitor binding seems to be affected by protein-degradation at room temperature concerning HL and LL material to a different extent (data not shown), the binding reaction was performed at 0 °C (Fig. 2). We got binding constants of $K = 0.049 \pm 0.004$ and 0.046 ± 0.004 μmol·l^{-1} for HL and LL material, respectively. The K-values at room temperature where much higher (around 0.13 μmol·l^{-1}), indicating a reduced affinity between the inhibitor and the inhibitor-binding site.

Table II. Distribution of Chl (%) in the Chl-protein complexes of *Sinapis alba* plants grown under HL (60 W·m^{-2}) and LL (6 W·m^{-2}) conditions. The difference to 100% is free Chl.

Component	HL	LL
CP a	13.5 ± 1.2	11.2 ± 0.9
CP I + Ia	20.2 ± 1.7	18.9 ± 1.1
LHCP	39.1 ± 1.7	44.5 ± 1.8

The results of Q_B protein determination at 0 °C (see Table I) are significantly different from those of the P-680 and Q_A determinations, respectively. Thus, only the results of Q_A and P-680 measurements are summarized as PS II content.

The P-700 content per Chl is independent from growth under different light conditions (Table I). In HL-adapted chloroplasts there are equal amounts of PS I and PS II centers. However, under LL conditions the PS I/PS II ratio is 1.5 which is due to the reduction of the PS II content per Chl.

The concentration of Cyt *f* and the size of the PQ pool are also strongly decreased under LL conditions as likewise demonstrated in Table I.

The results of the SDS-PAGE investigations are shown in Table II. CPa and CP I + Ia represent the reaction center complexes of PS II and PS I, respectively, while the LHCP$^{1-3}$ bands contain the light-harvesting Chl a/b-protein. A marked increase in light-harvesting Chl and a relative decrease in CPa content was found upon LL-acclimation.

Discussion

The results of our investigation clearly demonstrate that the electron transport components of mustard chloroplasts exhibit variable stoichiometries both with regard to one another and with regard to total Chl which strongly depend on the light intensity available during growth. While under HL conditions PS I and PS II exist in fairly equal amounts, PS II content is considerably decreased in LL-adapted plants, which is not the case for PS I. These results correspond well to the findings of Sampath and Kulandaivelu [8] and Leong and Anderson [9]. Comparable results concerning the functional manganese content of HL and LL thylakoids are reported [23].

With regard to our electrophoretical examinations (Table II), data of Lichtenthaler *et al.* [24] and Leong and Anderson [25, 26] show similar tendencies concerning the CPa/CP I + Ia ratio and also reveal a relative increase in light-harvesting Chl under LL conditions. Compared to HL-data, a somewhat higher CPa/P-680 ratio possibly hints at a slightly increased PS II attached antenna size in LL-grown plants whereas the size of the PS I attached antenna seems to remain fairly constant during the light adaptation (see Table I and II).

Doubtless, there is a considerable increase in the size of the functional PS II antenna (CPa + LHCP).

With regard to our results summarized in Table I and considering the lateral heterogeneity in the distribution of the different redox components in the thylakoid-membrane (see [27]), we propose functional models of the organization of the pigment system and the electron transport chain in HL and LL thylakoids of *Sinapis alba* (Figs. 4 and 5). They demonstrate the localization of the redox systems in

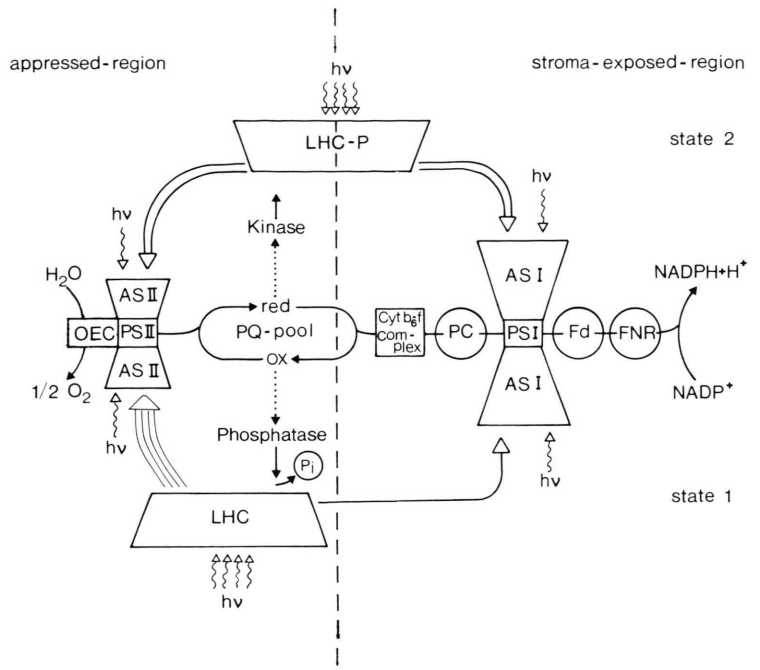

Fig. 4. Tripartite organization of the photosynthetic unit in HL thylakoids of *Sinapis alba*. The localization of the redox systems in different membrane regions and the different sizes of the attached antennae of PS I and PS II, respectively, are taken into consideration.
AS I = antenna system I, AS II = antenna system II, LHC = light-harvesting chlorophyll *a*/*b*-protein, LHC-P = phosphorylated light-harvesting chlorophyll *a*/*b*-protein, Fd = ferredoxin, FNR = ferredoxin-NADP$^+$ reductase, PC = plastocyanin, OEC = oxygen evolving complex, ...→ activation of an enzyme system, → ⇒ ⇛ direction and relative extent of energy distribution. The stoichiometries of the redox components are relative to the Cyt b_6-f complex.

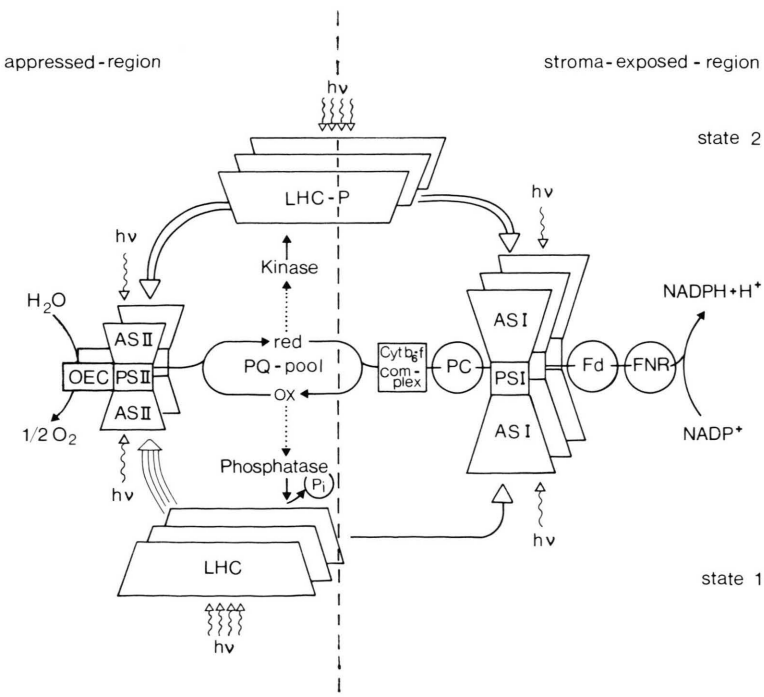

Fig. 5. Multipartite model of the photosynthetic unit in LL thylakoids of *Sinapis alba*. Symbols as in Fig. 4. Note that the reduction in PS II content per Chl leads to an increased functional antenna size (CPa + LHCP) for each of the PS II reaction centers, which is not the case for the PS I reaction centers. The stoichiometries of the redox components are relative to the Cyt b_6-f complex.

different membrane regions as well as the relative increase of light harvesting Chl per PS II reaction center under LL conditions. The feedback mechanism controlling the distribution of excitation energy between PS II and PS I by reversible phosphorylation of the LHCP is presented according to Staehelin and Arntzen [28]. State 1 designates the preferential association of light-harvesting protein to PS II while state 2 indicates a more even distribution between both photosystems. The models also demonstrate a large difference in the size of the photosynthetic unit, which is defined as the number of Chl molecules per electron transport chain or per Cyt *f* [4].

Under LL conditions a marked decrease in PS II content was observed, while the PS I concentration per Chl varied to a much lesser extent. The result is a decreased PS II/PS I reaction center ratio in LL-adapted plants. The decrease in P-680 concentration together with a simultaneous increase in light-harvesting Chl leads to a considerable enhancement in the absorbing capacity per PS II reaction center.

So far, these results are good in line with the work of other investigators and they are furthermore a clear demonstration of the ability of chloroplasts to adapt extensively to their environmental conditions.

This is in contrast to the proposals of Whitmarsh and Ort [29, 30], who used methods similar to ours. They found an invariant stoichiometry of the electron transport complexes and a constant antenna size (Chl : PS II : PS I : Cyt $f = 600 : 1 : 1 : 1$) in the chloroplasts of spinach plants, grown under different seasonal and geographical conditions.

The enormous increase of the photosynthetic unit size (Chl molecules per Cyt *f*) in LL thylakoids has to be seen as a question of economics. Under LL conditions – where light is the limiting factor of photosynthesis –, plants do not need as high an enzyme content as HL plants. The decrease of electron transport components, of the coupling factor, and of the enzymes of the Calvin cycle relatively to the Chl content is certainly an essential relief for the energy metabolism of LL plants (see [31]). To our view, adaptation to LL conditions appears to be first of all a question of the economical use of the available light energy including a refinement of energy trapping.

Interestingly, concerning the performance of the photosynthetic apparatus, the effects of light intensity seem to be somewhat different from the effects of light quality. In contrast to the adaptation effects under LL conditions, plants grown under far red enriched light show an increased PS II/PS I ratio compared to plants that are grown under far red depleted light [10, 12, 32]. Similar effects are produced by blue and red light [11, 33].

It has to be emphasized that a natural sun versus shade adaptation is due both to light quality and to light quantity. It is yet not clear wether the one or the other effect is predominantly responsible for the adaptative response of higher plants to natural sun and shade conditions.

A somewhat intriguing result of our investigation is the behaviour of the Q_B protein. Using the determination of the maximum concentration of atrazine binding sites (*i.e.* Q_B protein concentration) as a quantitative method of measuring PS II [9, 11, 33], a 1 : 1 stoichiometry between P-680 and Q_B protein should be expected. In fact we found the same HL/LL ratio of 1.4 (Table I) for all these PS II components, indicating a fixed stoichiometry, but there were differences up to 40% concerning the concentrations per Chl between P-680 and Q_A on the one and Q_B protein on the other hand. Similar effects are reported by Denneberg and Jursinic [34], who found up to 80% more atrazine binding sites per Chl than P-680. Thus we conclude that PS II determination based on the atrazine binding assay is essentially suitable for comparative and qualitative studies, but that it is probably not a reliable quantitative PS II determination method.

Acknowledgements

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[1] O. Björkman, N. K. Boardman, J. M. Anderson, S. W. Thorne, D. J. Goodchild, and N. A. Pyliotis, Carnegie Inst. Washington Yearb. **71,** 115 (1972).
[2] N. K. Boardman, Ann. Rev. Plant Physiol. **28,** 355 (1977).
[3] H. K. Lichtenthaler, C. Buschmann, M. Döll, H.-J. Fietz, T. Bach, U. Kozel, D. Meier, and U. Rahmsdorf, Photosynth. Res. **2,** 115 (1981).
[4] A. Wild, Ber. Deutsch. Bot. Ges. **92,** 341 (1979).
[5] H. Grahl and A. Wild, in: Environmental and Biological Control of Photosynthesis (R. Marcelle, ed.), p. 107, Dr. W. Junk Publishers, The Hague 1975.
[6] O. Björkman, in: Encyclopedia of Plant Physiology, New Series, Vol. 12A (O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, eds.), p. 57, Springer Verlag, Berlin 1981.
[7] A. Melis and J. S. Brown, Proc. Natl. Acad. Sci. USA **77,** 4712 (1980).
[8] P. Sampath and G. Kulandaivelu, Photosynth. Res. **4,** 351 (1983).
[9] T.-Y. Leong and J. M. Anderson, Photosynth. Res. **5,** 117 (1984).
[10] A. Melis and G. W. Harvey, Biochim. Biophys. Acta **637,** 138 (1981).
[11] T.-Y. Leong and J. M. Anderson, Biochim. Biophys. Acta **766,** 533 (1984).
[12] R. E. Glick, S. W. McCauley, and A. Melis, Planta **164,** 487 (1985).
[13] A. Wild, J. Belz, and W. Rühle, Planta **153,** 308 (1981).
[14] R. Ziegler and K. Egle, Beitr. Biol. Pflanz. **41,** 11 (1965).
[15] R. Emerson and W. Arnold, J. Gen. Physiol. **16,** 191 (1932).
[16] U. Schreiber, Photosynth. Res. **4,** 361 (1983).
[17] S. Malkin and E. Kok, Biochim. Biophys. Acta **126,** 413 (1966).
[18] W. Tischer and H. Strotmann, Biochim. Biophys. Acta **460,** 113 (1977).
[19] T. Hiyama and B. Ke, Biochim. Biophys. Acta **267,** 160 (1972).
[20] D. S. Bendall, H. E. Davenport, and R. Hill, Methods Enzymol. **23,** 327 (1971).
[21] A. Wild, B. Krebs, and W. Rühle, Z. Pflanzenphysiol. **100,** 1 (1980).
[22] L. Schladt, Diplomarbeit, FB Biologie, Univ. Mainz 1982.
[23] A. Wild, M. Stühn, and W. Rühle, Photosynth. Res. **2,** 105 (1981).
[24] H. K. Lichtenthaler, G. Kuhn, U. Prenzel, C. Buschmann, and D. Meier, Z. Naturforsch. **37c,** 464 (1982).
[25] T.-Y. Leong and J. M. Anderson, Biochim. Biophys. Acta **723,** 391 (1983).
[26] T.-Y. Leong and J. M. Anderson, Photosynth. Res. **5,** 105 (1984).
[27] W. Haehnel, Ann. Rev. Plant Physiol. **35,** 659 (1984).
[28] L. A. Staehelin and C. J. Arntzen, J. Cell. Biol. **97,** 1327 (1983).
[29] J. Witmarsh and D. R. Ort, in: Advances in Photosynthesis Research, **Vol. 3,** (C. Sybesma, ed.), p. 231, Dr. W. Junk Publishers, The Hague 1984.
[30] J. Witmarsh and D. R. Ort, Arch. Biochem. Biophys. **231,** 378 (1984).
[31] W. Rühle and A. Wild, Naturwissenschaften **72,** 10 (1985).
[32] A. Melis, J. Cell. Biochem. **24,** 271 (1984).
[33] T.-Y. Leong, D. J. Goodchild, and J. M. Anderson, Plant Physiol. **78,** 561 (1985).
[34] R. J. Dennenberg and P. A. Jursinic, Biochim. Biophys. Acta **808,** 192 (1985).