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THE RELATION BETWEEN THE PHOTOCHEMICAL ACTIVITY OF PS I, PS II AND THE STATE TRANSITIONS IN THE GREEN ALGA *MOUGEOTIA*, STRAIN AICB 560

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Abstract: The photosystems activation in state transitions in the presence of some specific inhibitors, as well as the emphasis of the changes in the redox state of electron carriers with the involvment of xanthophyll cycle in the response at light, is presented in the green alga *Mougeotia sp*. The state transitions were obtained by excitation with light *1* (over 700 nm) (*state 1*), light *2* respectively (620 nm) in aerobic conditions and in the presence of FCCP (*state 2*). The light *1* maintained a reduced state at the level of electron carriers with diminishing of nonphotochemical dissipation of excitation energy, correlated with violaxanthin de-epoxidase. In the presence of DCMU the plastoquinone reduced state diminished, the oxidation state being installed at the level of electron carriers. The light *2* increased the fraction of PS II opened reaction centers, respectively increased the reduced state and consequently intensified the nonphotochemical dissipation of excitation energy at the antenna level, alternating the oxido-reduction state at the level of electron carriers. The light *2* together with DCMU, DBMIB and chloramphenicol, generally maintained a reduction state at the level of electron transport chain. In *state 2*, the PS II activity was more inhibited by light than in *state 1*. The state transitions $S_1 - S_2$ produce changes in the internal structural organization of photosystems.

Keywords: cytochrome b_6f complex, cyclic electron transfer, nonphotochemical dissipation of excitation energy, photooxidative stress, photosystems, redox state, state transitions, signal transduction, violaxanthin de-epoxidation

Introduction

The oxygenic photosynthetic organisms possess a photoregulatory mechanism which equilibrates the energy of the absorbed light between the two photosystems PS II and PS I. This mechanism, named the *state 1* - *state 2* transition, is considered an adaptation for the efficient usage of light energy in conditions of light limitation [21,24] and it was first time observed by studing the fluorescence and the O_2 evolution in algae [3]. The state transition implies a reversible redistribution light capture antenna (LHC II) between PS I and PS II and the optimization of light energy usage in photosynthesis with the involvement of cyclic electron flow [9]. The state transitions can be explained by excitation energy transfer from PS II to PS I, requering the physical proximity of the two photosystems [23], or by absorbtion changes due to the migration of LHC II [4, 24]. The state transition was described as a chromatic adaptation on a short period of time, which permit the plants and algae to respond to changes in the spectral quality of light [9].

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The *state 1 - state 2* transition is produced by LHC II phosphorylation through a protein kinase linked to the thylakoidal membrane, activated by plastoquinone (PQ) reduction, either by PS II or by other secondary metabolic processes, and is inactivated through PQH_2 oxidation by PS I. P-LHC II dephosphorylation is assured by a phosphatase considered to be always active [1]. The inhibition of LHC II phosphorylation by intense light was interpreted in terms of conformational changes taking place in LHC II molecule, preventing the phosphorylation.

The cytochrome b_6f complex plays a key-role in the redox signal transduction from plastoquinol to kinase. This was proved in the mutants of *Chlamydomonas reinhardtii* laking the cytochrome b_6f complex, where the state transitions do not take place [25]. The first step of signal transduction is the link of PQH_2 to the quinol binding site (Q_0), from the cytochrome b_6f complex [22]. The conformational changes take place in the Rieske subunit of the complex, after the link of PQH_2 at Q_0-site, playing an essential role in LHC II kinase activation [8]. The activation signal generated on the lumenal side of thylakoids where Q_0 is located, requires a transduction through the membrane bilayer, because the active site of the kinase is situated on the stromatic side of the membrane. The nature of the elements involved in signal transduction is not well known. It was shown that the tetrapyrolic ring of the chlorophyll molecule present in the cytochrome complex is exposed toward the lipidic layer, and the phytol chain is localized much more profund on the interior of the complex, where possibly to interact with the quinol at Q_0-site. The chlorophyll molecule can offer thus a direct way to signal the quinol bond to Q_0-site toward a periferic region of the complex, where the kinase is thought to be linked. The region where the chlorophyll ring is exposed toward the lipid phase is localized in the vicinity of the proposed area of kinase linking to cytochrome b_6f on the basis on functional analysis in *Chlamydomonas* mutants [26].

Little is known about the activity of PS I and PS II in *state 1* and *state 2*. In algae, the state transition is much more dominating as compared to plants: 85% of LHC II is implicated in this process in algae, comparing with only 25% in plants [1]. The LHC II complexes in the state transition increase the performance of PS I and may represent a mechanism which permit the swich between the linear electron flow and the cyclic flow around PS I [20]. In the present paper the photochemical activity in *state 1* and *state 2* was studied in the presence of some specific inhibitors as well as the highlight of changes in the redox state of electron carriers, with the implication of xanthophyll cycle in the reaction to light.

Materials and Methods

The green alga *Mougeotia* sp. Agardh (AICB 560) belongs to the Culture Collection of Algae of the Institute of Biological Research Cluj-Napoca (AICB) [6]. The strain AICB 560 was grown for 23 days in Bold culture medium (BBM), with continuous air stirring and continuous illumination with 630 $\mu mol.m^{-2}.s^{-1}$.

Growth process.

The growth of algae was estimated by the growth curve obtained on the basis of $\Delta \log_2$ of optical density of suspension [19].

At the end of the growth period it was determined the amount of accumulated biomass as well as its content in assimilatory pigments and in cellular proteins. The chlorophyll content (chlorophyll a and b) was estimated spectrophotometrically on the basis of specific absorbtion coefficients [2]. The proteins were measured with the method of Lowry *et al.* [16].

The treatment with light and inhibitors

The PAR (phoposynthetic active radiation) of 5000 $\mu mol.m^{-2}.s^{-1}$ was applied for 60 minutes, at room temperature, in the presence of some specific inhibitors: 300 μM DCMU 3(3 – (3,4-diclorophenyl)-1,1-dimethylurea), 20 mM DBMIB (2,5-dibromo-3-methyl-6-izopropyll-p-benzoquinone), 230 μM cloramphenicol şi 2 μM FCCP (carbonylcyanide-p (trifluorometoxy)-phenylhydrazone). As light source halogen light bulbs of 500 W were used. The state transitions

were obtained by excitation of photosystems with different lights: *state 1* – light *1* (over 700 nm) far-red, emitted by the combination of red and blue filters at 470 $\mu mol.m^{-2}.s^{-1}$ intensity which activated PS I, respectively *state 2* – light *2* (620 nm) specific to PS II, emitted by the red filter with the intensity of 2300 $\mu mol.m^{-2}.s^{-1}$. The emission intensities were obtained by passing the 5000 $\mu mol.m^{-2}.s^{-1}$ light through the combinations of these filters.

The pigments analyses

For the analysis of pigmentary components, the algal suspension was ground and pigments extracted with acetone in the presence of $CaCO_3$. The chlorophyll content (chlorophyll a and b) was estimated spectrophotometrically on the basis of specific absorbtion coefficients [2].

The analyses of carotenoids by HPLC

The algal suspension was saponified with a solution of 30% KOH in ethanol, at room temperature for 24 hours. The carotenoids were extracted with diethyleter, and the extract was repeatedly rinsed with water and then evaporated. The resulted residue was eluted in 5 ml ethyl acetate and investigated by High Performance Liquid Chromatography (HPLC). The analysis was performed for the two systems:

- System 1 – for carotenoids identification: it consists of Altex 110 A (Kontron AG) pumps, Altex mixing room, Altex gradient automat controller, Waters 990 photodiode-array detector and Waters 990 soft for data acquisition and processing.

- System 2 – for quantitative measurements: the Agilent 1100 system contains a degasser, the Agilent G1311A quaternary pump system and the Rheodyne injector equipped with a 20 µl curl, a EC 250/4,6 Nucleosil 120-5 C18 column, an UV/VIS Agilent G1314A detector and a Chemstation Agilent software. The used gradient: 0 min: 10% B / 90% A; 20 min: 70% B / 30% A (A is represented by the acetonitrile: water = 9:1 mixture, while B is the ethyl acetate). The chromatograms display the major carotenoids function of the retention period. The separations monitoring was accomplished at 450 nm, 35^{o}C and 144 barr pressure. The quantitative analysis of carotenoids was made by the external standard method [12]. Violaxanthin de-epoxidation, respectively the light dependent accumulation of zeaxanthin was analyzed *in vivo* by measuring the absorption changes at 505 nm (ΔA_{505}) [27]. The redox state of the P_{700} reaction center of PS I was analysed by the absorption changes at 710 nm [17]. The redox state of the quinone acceptor of PS II was determined on the basis of absorption changes at 320 nm. The redox state of cytochrome b_6f was determined after Joliot and Joliot [14].

Chlorophyll fluorescence analysis

The chlorophyll fluorescence was measured with PAM-210 fluorometer described by Schreiber *et al.* [18]. The fluorescence parameters and the quenching analysis were conducted by the saturation pulse method. The quantum yield of the photochemical energy conversion was determined using the equation Yield = $\Delta F/F_M$, and the ratio F_V/F_M ($F_V/F_M = F_M-F_0/F_M$) that shows the photochemical quantum yield of the closed PS II reaction centers.

Results and Discussion

The exponential growth of *Mougeotia* cultures at 630 $\mu mol.m^{-2}.s^{-1}$ medium intensity light, is illustrated in figure 1. The cultures presented relatively exponential growth, with an exponential growth rate (R) = 0.132, a medium growth rate (R_m) = 0.053 and a generation time (g) = 7.58.

The parameters of exponential growth show a slow process according to the growth rate, with long generation time. The growth of culture is based on a linear correlation, with a high correlation coefficient. The accumulated biomass contains a high quantity of proteins and assimilatory pigments. The ratio between the photosythetic compounds shows normal values (Table 1).

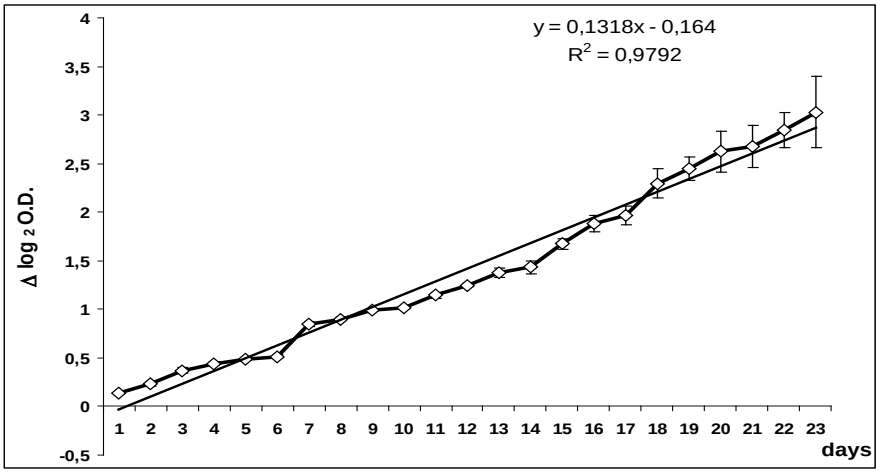

Fig. 1: The exponential growth curve of the algal suspension

The state transitions took place by photosystems excitement with two types of light. Light *1* with a wavelenght over 700 nm (far-red), with a 470 μmol.m^{-2}.s^{-1} intensity, which activated photosystem PS I, was used to induce *state 1*. This light was obtained by the combination of red-blue filters, whose transmission presents a maximum at 365 nm in blue spectrum and another dominant maximum at over 700 nm (fig. 2 A). Light *2*, which activates photosystem PS II, was emitted with a red filter at 2300 μmol.m^{-2}.s^{-1} intensity, was used to induce *state 2*. The transmission spectrum for light *2* presents a maximum in the blue range at 355 nm, another maximum at 545 nm, and the dominant maximum at 620 nm (Fig. 2 B). *State 2* was obtained in aerobiose in the presence of FCCP ionophore [9].

The algal suspension was incubated for one hour in dark and then exposed for another hour at photosystems excitation light, for the measurement of chlorophyll fluorescence. In the control variant a high quantic efficiency and quantic yield was recorded, as well as a slightly nonphotochemical dissipation of excitation energy (Fig. 3). By exposure to light *1*, specific to PS I, it was registered the decrease of F_0 and F_m proportionably with the exposure time (Fig. 3, V_1). The quantic efficiency and quantic yield increase compared with the control, showing constant values, with an important decline in the first 30 minutes of exposure to light *1*. The increase of quantic yield can be attributed to PS I activity in the specific light (far-red). The high values of photochemical coefficient (q_P) shows that a high ratio from the excitation energy was used in photochemistry, reduction of the number of Q_A primary and secondary acceptor, in oxidized and reduced state.

The nonphotochemical coefficient (q_N) showed a decreased activity of nonphotochemical excitation energy dissipation, important part of this energy was directioned from the antenna to the reaction centers.

The exposure of algal suspensions at light *1* in the presence of photosynthetic inhibitor 300 μM DCMU, leaded to the fluorescence increase and to the decrease of quantic yield (fig. 3, V_2). The photochemical coefficient (q_P) decreased, fact denoting the decrease of photochemical quantum conversion, as well as of decreasing the plastoquinone reduction intensity. The quantic yield now defines the photochemical activity of PS I, because PS II is inhibited. The nonphotochemical dissipation of excitation energy was zero.

Exposing the algal suspension to light *2* and in the presence of 2 μM FCCP ionophore, the fluorescence F_0 and F_m increased, conducting to the increase of photochemical efficiency of PS II closed reaction centers as well as the quantic yield and the photosynthetic electron transport (Fig. 3, V_3). The ionophore FCCP enables the pH equilibration between the cellular components [9] or can play the role of electron transport decuplant [10]. The photochemical coefficient (q_P) increased, showing the increase of the fraction of PS II open reaction centers, and the increase of

reduction state, respectively. As a result, the nonphotochemical excitation energy dissipation (NPQ) intensified at PS II antenna level. NPQ increases with the light intensity [11].

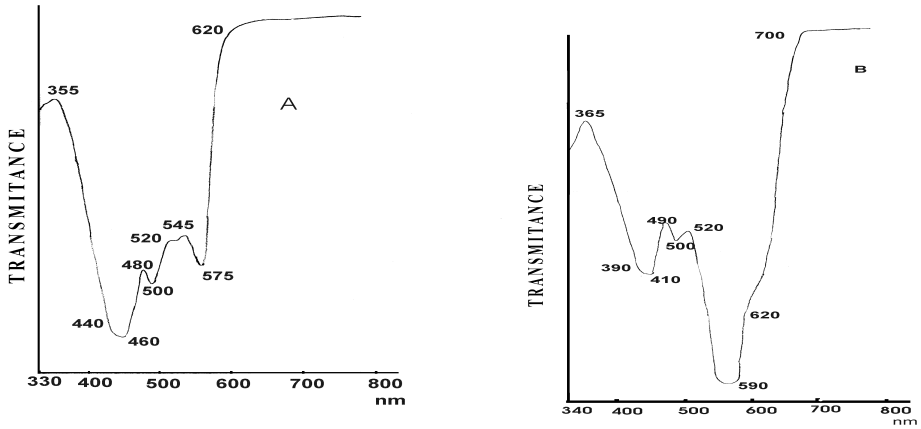

Fig. 2: The emission spectrum of light *1* and *2* for the PS I and PS II excitation

In the presence of DCMU and FCCP, light *2* incresed the basal fluorescence F_0, fact conducting to the diminishing of variable fluorescence F_V (Fig. 3, V_4). The photochemical efficiency of PS II as well as the quantic yield of photosynthesis decreased, being accompanied by the decrease of the fraction of open reaction centers, expressed by the photochemical coefficient (q_P). The quite equal values between F_m and F'_m express a lack of energizing process at the level of thylakoidal membrane. Using the DBMIB inhibitor the basal fluorescence F_0 decreased with the exposure time, conducting to the increase of F_v. The decrease of fluorescence is due to the increase of photochemical rate and the excitation energy dissipation rate at the level of photosystem antenna (Fig. 3, V_5). DBMIB inhibits the photosynthetic electron transport from plastoquinone to cytichrome b_6f [5, 10]. The efficiency of the energy absorption of open reaction centers (F_V/F_m) increased significantly, being in direct relationship with the quantic yield of electron transport chain and with the photochemical coefficient (q_P).

The nonphotochemical dissipation of excitation energy decreases with exposure time. DBMIB becomes an inhibitor of state transitions only after the cells have been illuminated [8].

When the light is absorbed in excess, higher than as it can be used in photochemistry, damages of photochemical apparatus take place. The lost of activity was named photoinhibition and conducts to the degradation of D_1 subunit of PS II reaction centers. Treating the algal suspension with 230 μM chloramphenicol which is a protein inhibitor, F_0 increased compared with the control, and F_m decreased, conducting to the decrease of F_v. The photochemical efficiency and the quantic yield decreased, showing the close down of PS II reaction centers. The photochemical coefficient has been also reduced, showing that a saturation in the photochemical conversion of quanta exists, fact conducting to the increase of nonphotochemical dissipation of excitation energy (q_N). (Fig. 3, V_6).

In *state 2*, the PS II activity was more inhibated as compared with *state 1* because a substantial degradation of D_1 took place in *state 1*. This suggest that the photoinhibition is installed by the creation of an intermediate state in which PS II is inactive, but D_1 protein is still intact. The accumulation of this state is high in *state 2* in which only the cyclic photosynthetic electron flow is active, but not a single electron flows between the PS II and the cytochrome b_6f. This permits the reparation of PS II lesions due to the cell ability to maintain the high rate of ATP synthesis during the electron flow to PS I, a physiological trait important in protecting the photosynthetic apparatus from the excess light as well as from other abiotic stress conditions [7].

The accumulation of chlorophyll a was intensified in the majority of treatments, excepting the V_2 variant in which there were obtained more diminished quantities compared with

the control (Table 1). It was observed the increase of chlorophyll a concentration by exposure to light *1* in *state 1*.

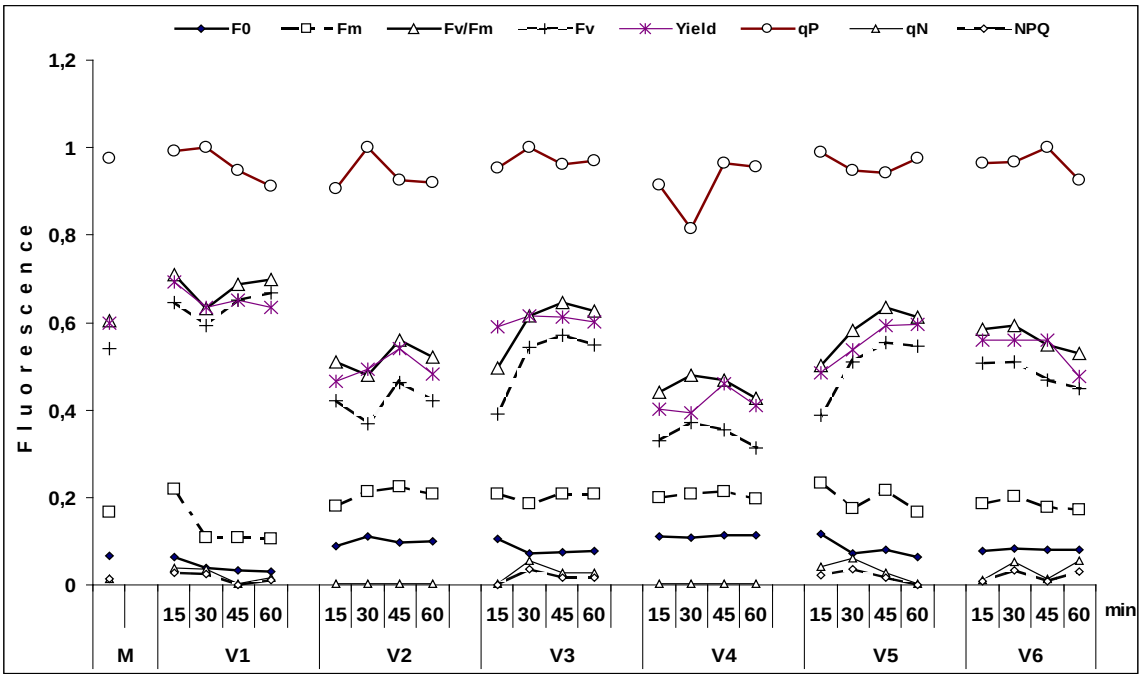

Fig. 3: The chlorophyll fluorescence of the experimental samples: M-control; V_1-light *1;* V_2-light *1* +DCMU; V_3-light *2* _FCCP; V_4-light *2* +FCCP+DCMU; V_5-light *2* +FCCP+DBMIB; V_6-light *2* +FCCP+ chloramphenicol

In the presence of DCMU, which blocks the transfer of electrons between PS II acceptors, light *1* cannot sustain the photosynthetic rate at the same level in the control (V2). In *state 2*, light *2* stimulated the chlorophyll a accumulation as compared with the control values. Chlorophyll b concentration decreased in the majority of the variants, excepting V_2 where it has been observed a slight stimulation.

The major carotenoids separated and identified by HPLC are shown in table 2 and in figure 4. The content in carotenoids increased in the case of all variants, comparing with the control values. The carotenoid concentration incresed in the case of V_1 and V_2, respectively in *state 1*. The lutein quantity increased in all variants. From the components of xanthophyll cycle, the violaxanthin concentration increased, but antheraxanthin increased only withV_2 in *state 1*, in the presence of DCMU. Zeaxanthin presented low levels, with the exception of V_3 variant. The presence of violaxanthin in high quantity and the diminishing of zeaxanthin stand for the noninvolvement of xanthophyll cycle because the photochemical activity does not requires the nonphotochemical diminishing of excitation energy. The carotenoids decrease and the increase of neoxanthin highlights the changes at the level of reaction centers. The presence of these carotenoids function of the retention time is shown in the chromatogrames in figure 4.

The plants grown in reduced light preserve the maximum efficiency of PS II open centers. In excess light the photochemical efficiency (Fv/Fm) decreases with 35% and is corellated with over 50% of the xanthophyll cycle carotenoids which are de-epoxidated in the form of A+Z. The loss of PS II efficiency is associated with the dissipation of energy dependent of the xanthophyll cycle in antenna [11]. The carotenoids represent important quenchers of singlet oxygen and reduce its form by chlorophyll deactivation in triplet excitation state [15].

Table 1: The content of algal biomass assimilatory pigments (related to g of dry substance)

Pigment	M	V_1	V_2	V_3	V_4	V_5	V_6
chlorophyll a (x 10^{-5})	2.53	3.42	2.06	2.79	3.03	3.24	2.82
chlorophyll b	0.45	0.41	0.42	0.41	0.40	0.41	0.39
Neoxanthin	19.26	240.11	16.56	32.30	117.41	46.63	65.67
Violaxanthin	122.32	158.17	185.71	176.42	134.30	141.39	139.12
Lutein-5,6-epoxid	14.84	0.73	13.37	0.89	0.83	0.77	4.44
Anteraxanthin	52.44	42.45	78.99	47.55	15.69	38.63	17.75
Lutein	210.59	315.33	347.34	341.33	303.25	309.02	331.31
Zeaxanthin	1.02	0.75	1.25	12.91	0.85	10.33	0.91
α-cryptoxanthin	1.05	0.77	1.28	4.74	0.87	0.82	0.94
α-carotene	10.78	15.95	15.89	0.98	9.90	0.84	0.97
β- carotene	52.82	92.50	79.43	58.65	54.00	43.77	33.85
9Z-β- carotene	17.25	13.56	17.21	16.62	0.9	0.84	0.96

chlorophyll a in mole;chlorophyll b in mole/mole of chlorophyll a; carotenoids in mole/mole of chloroplyll a

M-control; V_1-light *1*; V_2-light *1* +DCMU; V_3-light *2* _FCCP; V_4-light *2* +FCCP+DCMU; V_5-light *2* +FCCP+DBMIB; V_6-light *2* +FCCP+ chloramphenicol

Table 2:The retention periods for the identified carotenoids (in minutes)

Nr.	Carotenoids	M	V_1	V_2	V_3	V_4	V_5	V_6
1.	Neoxanthin	2.688	2.871	3.913	2.870	2.885	4.530	4.251
2.	Violaxanthin	8.5	8.5	8.57	8.481	8.459	8.56	8.537
3.	Lutein-5,6-epoxid	9.685	9.683	9.765	9.667	9.637	9.765	9.742
4.	Anteraxanthin	10.014	10.012	10.11	9.992	9.963	10.107	10.086
5.	Lutein	12.691	12.699	12.819	12.664	12.64	12.815	12.796
6.	Zeaxanthin	13.039	13.041	13.168	13.012	12.982	13.168	13.149
7.	α-cryptoxanthin	15.951	15.969	16.069	15.941	15.898	16.094	16.08
8.	β - carotene-5,6 epoxid	16.9	-	17.045	16.853	16.853	17.023	-
9.	α - carotene	19.826	19.832	20.1	19.773	19.735	20.043	19.997
10.	β - carotene	20.272	20.278	20.571	20.214	20.175	20.508	20.459
11.	9Z - β - carotene	20.691	20.695	21.015	20.627	20.587	20.948	20.896
12.	15Z - β - carotene	21.306	21.313	21.653	21.237	21.199	-	21.525

The PS II redox state in state transitions was monitored by the measurements of absorbtion changes at 320 nm (Fig. 5). In *state 1*, light *1* absorbed by PS I, determines the maintanance of quinonic acceptor of PS II into a continuous redox state of reduction. In the presence of DCMU, the quinonic acceptor of PS II shifts from the oxidized state, in the first 30 minutes of exposure to light *1*, at the reduction redox state (V_2). In *state 2*, the reduction-oxidation state of PS II quinonic acceptor is variable during the exposure to light *2* (V_3). In the presence of DCMU, the reduction state is replaced with the oxidation state toward the end of exposure period (V_4). The DBMIB inhibitor brings the quinonic acceptor of PS II from the oxidation state to the reduction state by exposure to light *2* (V_5). Chloramphenicol which is considered an inhibitor of protein synthesis, mantained in a reduction state the quinonic acceptor of PS II during the exposure to light *2*.

The photochemical activity measured as stocked energy in photosystems in *state 1* and *2* changed significantly during the state transition due to the migration of the light harvesting complex of photosystem II (LHC II). Within the PS I and PS II action spectrum, the absorption peaks change their position because of the LHC II association/dissociation. The PS II activity was three times more intense or equal with PS I in *state 1* and *2*. The amplitude of the induced changes by coupling LHC II was more reduced in PS I comparatively to PS II, this explaining the reduced photochemical activity in *state 2* related to *state 1*. This may happen due to the

decrease of the transfer energy from the carotenoids to chlorophylls in LHC II when they are associated with PS I [21].

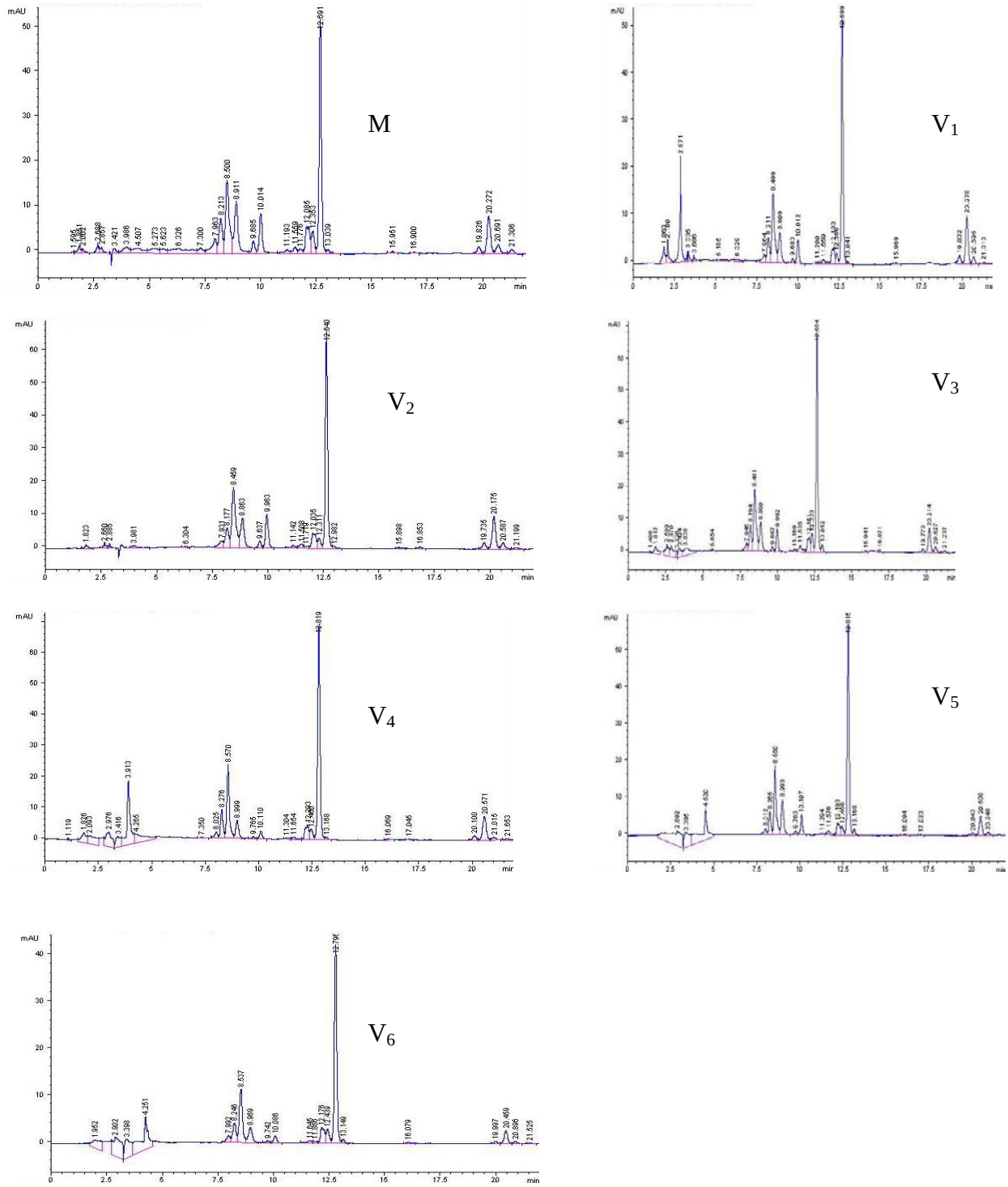

Fig. 4: The carotenoids separated by HPLC chromatogram.
M = control ; *State* 1: V_1=light *1* ; V_2 – light *1* + DCMU; *State 2*: V_3 – light *2* + FCCP; V_4 – light *2* + FCCP + DCMU; V_5 – light *2* + FCCP + DBMIB; V_6 – light *2* + FCCP + chloramphenicol

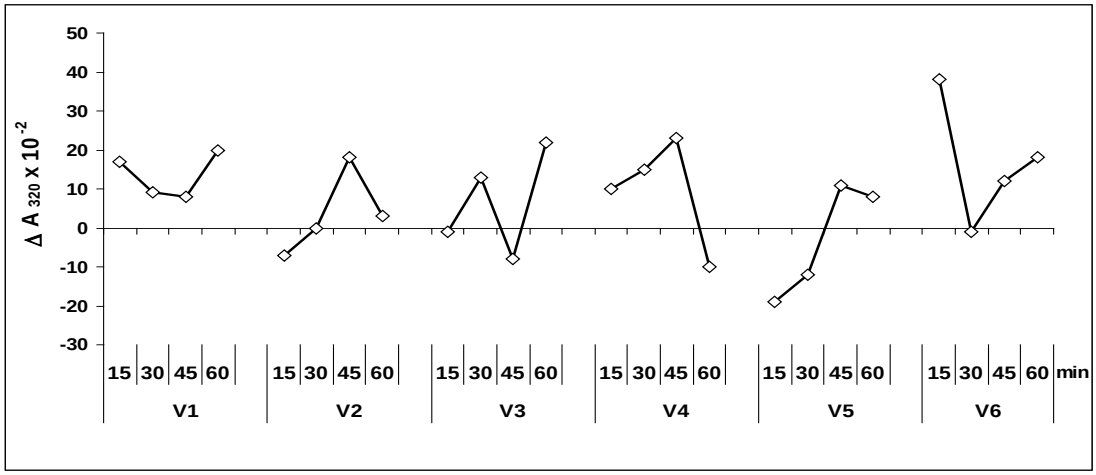

Fig. 5: The absorption changes at 320 nm regarding the PS II redox state
M = control; *State* 1: V_1=light *1*; V_2 – light *1* + DCMU; *State 2* : V_3 – light *2* + FCCP V_4 – light *2* + FCCP + DCMU; V_5 – light *2* + FCCP + DBMIB; V_6 – light *2* + FCCP + chloramphenicol

The redox state of the P_{700} reaction center of the PS I was analyzed by the absorption changes at 702 nm (Fig. 6). In the presence of *light 1* which is specific for PS I, the PS I reaction center switches from the oxidation state to reduction state due to the reception of electrons by the photosystem activation in *state 1* (V_1). By adding DCMU in the reaction medium, the redox state of the PS I reaction center, becomes usually oxidized, leading to the loss of the electrons used in various pathways around PS I (V_2). In the *state 2* which is specific for PS II, the PS I reaction center is maintained in a reduction state due to an electrons reception (V_3), even in the presence of DCMU (V_4). In the presence of DBMIB and chloramphenicol, the oxidation state of the PS I reaction center is dominant (V_5 and V_6).

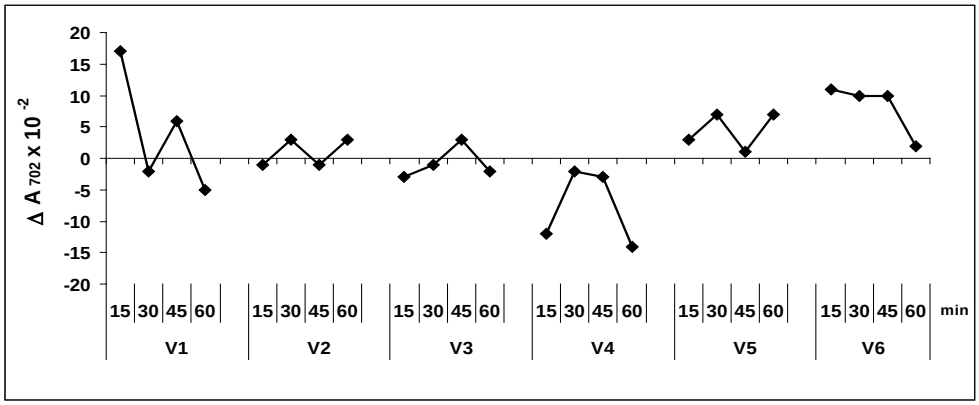

Fig. 6: The absorption changes at 702 nm regarding the PS I reduction state. *State* 1: V_1=light *1*; V_2 – light *1* + DCMU; *State 2*: V_3 – light *2* + FCCP; V_4 – light *2* + FCCP + DCMU; V_5 – light *2* + FCCP + DBMIB; V_6 – light *2* + FCCP + chloramphenicol

The redox of the cytochrome *b_6f* complex during the state transitions is presented in figure 7. In *state 1*, the cytochrome *b_6f* was maintained in a reduction state because *light 1* activates the PS I activity which supplies electrons through the cyclic flow (V_1). By adding DCMU, the state of the cytochrome *b_6f* becomes oxidized in the final exposure to *light 1* because the amount of electrons from PS II is reduced (V_2). During the *state 1*, the oxidation of

the cytochrome b_6f becomes dominant when exposed in *light 2* which induces a PS II specific activity and thus the amount of electrons flowing from the PS II enhances (V_3). In the presence of DCMU the *light 2* generates the reduction of the cytochrome b_6f which receives electrons only from PS I (V6). In the presence of DBMIB, which is a cytochrome b_6f specific inhibitor, the redox state oscillates between the reduced and the oxidized state during exposure to *light 2* (V_5).

The state transition was considered as being a mechanism for equalizing the absorption capacity of the two photosystems with the changes in the light spectral composition. The state transition describes the reversible association of the LHC II antenna complex with each photosystem: with PS II in the *state 1* and with PS I in the *state 2*. The cytochrome b_6f plays a role in the state transitions regulation and it establishes the bond between the linear and cyclic electron flows which follows the state transitions [9].

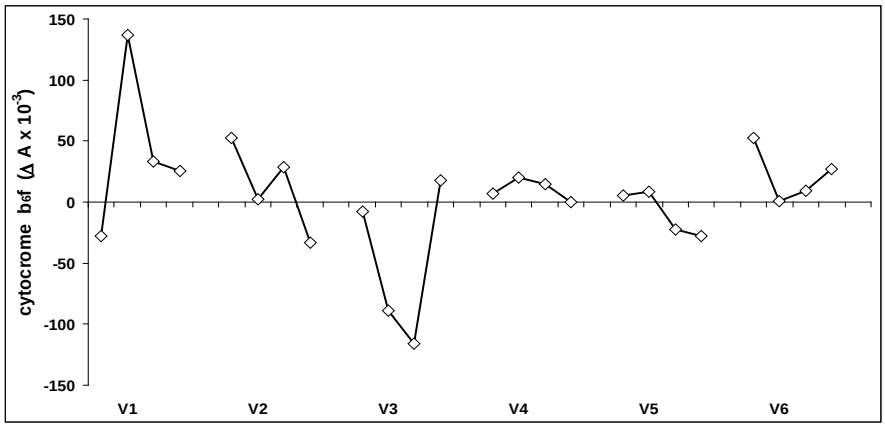

Fig. 7: The redox state of the cytochrome b_6f . *State* 1: V_1=light *1*; V_2 – light *1* + DCMU; *State 2*: V_3 – light *2* + FCCP; V_4 – light *2* + FCCP + DCMU; V_5 – light *2* + FCCP + DBMIB; V_6 – light *2* + FCCP + chloramphenicol

The xanthophyll cycle acts in the presence of light toward the zeaxanthin production as a result of the violaxanthin de-epoxidase activity, while in the dark the zeaxanthin epoxidation acts to produce violaxanthin. By monitoring the absorption changes at 505 nm epoxidation activity can be estimated (Fig. 8). During *state 1* the violaxanthin de-epoxidation has been intensified leading to the zeaxanthin accumulation, through excitation with *light 1*. By adding DCMU, the violaxnthin de-epoxidase has been reduced, the zeaxanthin became also reduced because of the PS II electrons blockage. During the *state 2* under the *light 2* the violaxanthin de-epoxidase activity was oscillatory (V_3). Together with DCMU, the *light 2* stimulated the violaxanthin de-epoxidation, but in the presence of DBMIB inhibited the de-epoxidation. The *light 2* and the chloramphenicol maintained the violaxanthin de-epoxidation at high values.

The $S_1 - S_2$ state transitions produce changes in the internal structural organization of photosystems. The energy redistribution between the two photosystems is emphasized by the absorption changes which may reflect the LHC II complexes phosphorylation and their mobility between the two photosystems [13].

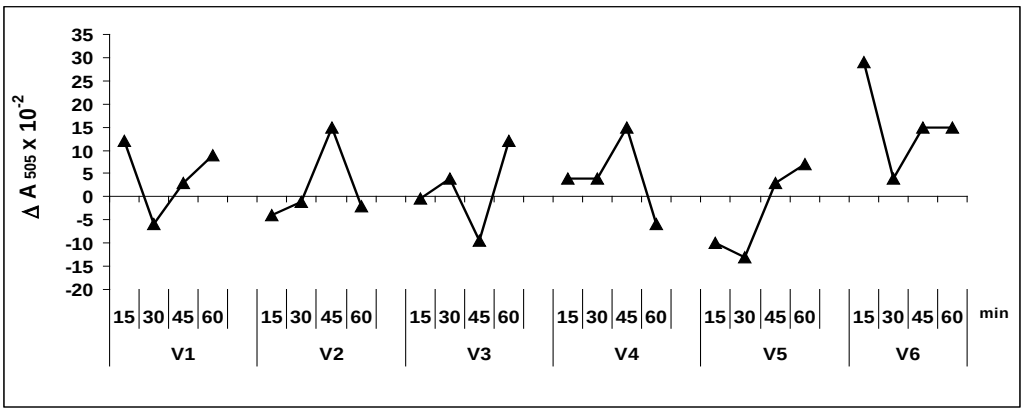

Fig. 8: The violaxanthin de-epoxidase activity during the state transitions. *State* 1: V_1=light *1* V_2 – light *1* + DCMU; *State 2*: V_3 – light *2* + FCCP; V_4 – light *2* + FCCP + DCMU; V_5 – light *2* + FCCP + DBMIB; V_6 – light *2* + FCCP + chloramphenicol

Conclusions

The *Mougeotia* cultures displayed an exponential growth with an exponential growth rate of (R) = 0,132, a medium growth rate of (R_m)=0,053 and a generation period of (g)=7,58.

By exposure to *light 1* which is specific for PS I, there was observed a decrease of F_0 and F_m related to the exposure time and to the quantic efficiency and quantic yield growth due to the PS I activation by far-red light which maintains a reduction state in the electrons transport. The photochemical coefficient has shown that the large proportion of the excitation energy was used in photochemistry, and the proportion of the primary and secondary Q_A acceptor in oxidized and reduced state has significantly decreased. The nonphotochemical coefficient (q_N) has shown a low activity of excitation energy nonphotochemical dissipation correlated with the violaxanthin de-epoxidase.

The *light 1* exposure in the presence of DCMU has lead to the fluorescence growth and to the decrease of the quantic yield together with the decrease of the conversion efficiency of the photochemical quanta decreasing the plastoquinone reduction, this facilitating the oxidation state at the level of the electrons transport.

The observed quantic yield defines the PS I photochemical activity because PS II is inhibited, and the violaxanthin de-epoxidase activity decreased.

The *light 2*, in the presence of the FCCP ionophore produced the F_0 and F_m enhancement, leading to the increase of the photochemical efficiency of the closed PS II reaction centers and also of the quantic yield using alternatively the oxido-reduction state in the electron transporters. The number of the open PS II reaction centers enhanced, as well as the reduction state of the photosystem and thus the nonphotochemical dissipation of the excitation energy at the antenna photosystem had been intensified determining a variable activity of the violaxanthin de-epoxidase.

The *light 2* together with DCMU produced the increase of the basic and the photochemical efficiency and the quantic yield decreased, as well as the proportion of the open reaction centers. At the level of the photosynthetic electron transporters the reduction state which activates the violaxanthin de-epoxidase has been achieved. The DBMIB uncoupler had positive effects on the photochemical activity alterning the oxido/reduction state of the electron transporters inhibating the violaxanthin de-epoxidase. In the presence of chloramphenicol the fluorescence parameters have been reduced and they have induced a reduction state to the electron transporters and to the enhancement of the nonphotochemical dissipation of the excitation energy which is correlated with the violaxanthin de-epoxidase activity. The chlorophyll a and carotenoids accumulation has been intensified in almost all the samples, comparatively to the control values.

In *state 2*, the PS II activity was more inhibited by light than in *state 1* because in this case the cyclic electron flow is active, and there is no electron that flows between PS II and the cytochrome b_6f. The photochemical activity was correlated with the redox state of the electron transport chain elements, and with the specific absorption changes. The $S_1 - S_2$ state transitions produce changes in the internal structural organization of photosystems. The energy redistribution between the two photosystems is emphasized by the absorption changes which may reflect the phosphorylation of the LHC II complexes and their mobility between the photosystems.

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RELAȚIA ÎNTRE ACTIVITATEA FOTOCHIMICĂ A FOTOSISTEMELOR PS I, PS II ŞI TRANZIȚIILE DE STARE LA ALGA VERDE *Mougeotia,* TULPINA AICB 560

(Rezumat)

În această lucrare este prezentată activitatea fotosistemelor în tranzițiile de stare în prezența unor inhibitori specifici precum şi evidențierea schimbărilor în starea redox a transportorilor de electroni cu implicarea ciclului xantofilic în reacția față de lumină.

Alga verde *Mougeotia sp*. Agardh (AICB 560), provine din Colecția de culturi de alge a I.C.B. Cluj-Napoca (AICB). Tulpina AICB 560 a fost crescută în soluția nutritivă Bold (BBM), în condiții de agitare continuă cu aer, iluminare continuă cu 630μmol.m $^{-2}$.s^{-1}s^{-1}, la temperatura de 22^{0}C. Durata de cultivare a fost de 23 de zile. Creşterea suspensiei algale s-a estimat prin trasarea curbei de creştere pe baza **Λlog$_2$** a densității optice. La finele perioadei de creştere s-a determinat acumularea de biomasă precum şi conținutul acesteia în pigmenți asimilatori şi în proteine celulare. Conținutul în clorofile (clorofila a şi b) şi proteine s-a estimat spectrofotometric, iar carotenoidele au fost analizate prin HPLC.

Intensitatea de lumină PAR (radiația activă fotosintetic) de 5000 μmol.m^{-2}.s^{-1} s-a aplicat timp de 60 minute, la temperatura camerei în prezența unor inhibitori specifici: 300 μM DCMU(3 –(3,4-diclorofenil)-1,1-dimetilurea), 20 mM DBMIB (2,5-dibromo-3-metil-6-izopropil-p-benzochinone), 230 μM cloramfenicol şi 2 μM FCCP (carbonilcianide-4(trifluoro-metoxi)-fenilhidrazone). Tranzițiile de stare s-au obținut prin excitarea cu lumina 1 (peste 700 nm) (*starea 1)*, respectiv, lumina 2 (620 nm) în condiții aerobe şi în prezența FCCP (*starea 2).* Intensitățile de emisie s-au obținut prin trecerea luminii de 5000 μmol.m^{-2}.s^{-1} prin combinația acestor filtre.

Lumina *1* a menținut o stare de reducere la nivelul transportorilor de electroni cu o diminuare a disipării nefotochimice a energiei de excitație corelată cu violaxantin de-epoxidaza. În prezența DCMU a scăzut reducerea plastochinonei instalându-se oxidare la nivelul transportorilor de electroni. Lumina *2* a mărit proporția centrilor de reacție deschişi ai PS II, respectiv a crescut starea de reducere şi implicit s-a intensificat disiparea nefotochimică a energiei de excitație la nivelul antenei alternând starea de oxido-reducere la nivelul transportorilor de electroni. Lumina *2* alături de DCMU, DBMIB şi cloramfenicol, în general au menținut o stare de reducere la nivelul lanțului transportor de electroni. În *starea 2*, activitatea PS II a fost mai inhibată prin lumină față de *starea 1*.

În *starea 2*, activitatea PS II a fost mai inhibată prin lumină față de *starea 1* deoarece acum este activ numai transportul ciclic de electroni fotosintetic, căci nici un electron nu curge între PS II şi citocromul b_6f. Activitatea fotochimică s-a corelat cu starea redox a componenților lanțului transportor de electroni în tranzițiile de stare, respectiv cu schimbărilor în absorbție specifice. Tranzițiile de stare $S_1 – S_2$ produc modificări în organizarea internă structurală a fotosistemelor. Redistribuția energiei între cele două fotosisteme este evidențiată semnificativ prin schimbările în absorbție care pot reflecta fosforilarea complexelor LHC II şi mobilitatea acestora între fotosisteme.

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