

## THE INDUCEMENT OF CHLOROPHYLL FLUORESCENCE IN STATE TRANSITIONS UNDER LOW TEMPERATURE IN THE *Mougeotia* ALGA, STRAIN AICB 560

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The alga was grown using Bold nutritive solution (BBM) with continuous air stirring and illumination with  $485 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , at  $22^{\circ}\text{C}$ , during exponential growth.  $F_0$  has decreased in *state 2* under preillumination and low temperature, and in *state 1*, in the presence of DCMU and methylviologen,  $F_0$  has been the same with the control value.  $F_m$  decreased in both *state 1* and *2* which lead to a decrease of the variable fluorescence. The  $F_v/F_m$  and the quantum yield decreased significantly in both redox states, highlighting the photoinhibition of the closed reaction centers. The excitation pressure decreased, and in the presence of DBIMB, it increased in *state 1*, which states the growth of the closed  $Q_A$  proportion.  $q_p$  has increased in *state 1* which emphasizes the growth of the opened  $Q_A$  proportion. The increase in the non-photochemical dissipation of the excitation energy is correlated with the excitation pressure.

The inducement of chlorophyll fluorescence with quenching analysis on a short period of time in *state 1* and *2* on whole cells marks out the proportionality ratio with the light harvesting antenna size.

*Key words:* Chlorophyll fluorescence; Low temperature; Photochemical activity; State transitions.

### INTRODUCTION

Organisms with oxygenic photosynthesis have a photoadjustment mechanism for redistribution of the absorbed light energy between the two photosystems PS I and PS II. This mechanism, called *state 1–state 2 transitions*, is thought to be a short term adaptation for efficient usage of light energy in limitation conditions<sup>2,18,22</sup>. The state transitions involves a reversible redistribution of the light harvesting antenna between PS I and PS II and optimizes the light energy used in photosynthesis through the cyclic electron current<sup>4,6,21</sup>.

In green plants *state 1* and *state 2* are induced by exposing the leaves at light 1 far-red which exceeds 715 nm and light 2 between 400–600 nm. In *state 2* are generated both the phosphorylation and decoupling of LHC II from the PS II<sup>4</sup>. LHC,

dissociated from PS II, migrate towards stromatic lamellas rich in PS I and couple with PS I due to conformational changes which appear in the protein after phosphorylation<sup>15</sup>.

*State 1* transition involves dephosphorylation and dissociation of LHC II from PS I and their migration to grana lamellas rich in PS II. The migration of LHC II to PS I by lateral diffusion (*state 1–state 2* transitions) is produced after phosphorylation of LHC II by a protein kinase bound to the thylakoid membrane and activated by the reduction of plastoquinone (PQ) through PS II or other secondary metabolic processes. In oxidative conditions, the kinase is inactivated through oxidation of PQH<sub>2</sub> by PS I and the LHC II are dephosphorylated by a phosphatase (*state 2–state 1* transitions) bound to thylakoids and permanently active<sup>1,7,9</sup>. After dephosphorylation, LHC II return to PS II.

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The cytochrome *b<sub>6</sub>f* complexes play a key role in sending the redox signal from plastoquinol to kinase<sup>23</sup>. The first step of signal transmission is the binding of PQH<sub>2</sub> to Q<sub>0</sub> place in the cytochrome *b<sub>6</sub>f* complexes<sup>20</sup>. Conformational changes are produced in the lumen of the complex's Rieske subunit after binding of PQH<sub>2</sub> to Q<sub>0</sub>, playing an essential role in the LHC II kinase activation<sup>8</sup>. The activation signal generated on the luminal side of the thylakoids where Q<sub>0</sub> is located needs to be transmitted through the bilayer membrane because the active domain of the kinase is located on the stromatic side of the membrane. In this way, the chlorophyll molecules can supply a direct way for transmitting the signal for quinol binding to Q<sub>0</sub> place to a marginal region of the complex, where the kinase is supposed to be located. The region where the chlorophyll ring is exposed towards the lipidic phase is located near the area proposed as kinase- cytochrome *b<sub>6</sub>f* binding site<sup>24</sup>. In the state transitions LHC II complexes increase the PS I performance and can represent a shifting mechanism between the linear and cyclic electron current around the PS I<sup>18</sup>.

According Finazzi *et al.*<sup>7</sup>, *state 1* is induced by incubating the cells in dark with continuous agitation, and *state 2* is obtained by dark incubation in anaerobic conditions through argon pumping. Incubating the algae in conditions which promote *state 2* – anaerobic or aerobic conditions and FCCP – generate an electron source passing through the cytochrome *b<sub>6</sub>f*. Reactivation of the linear electron current between PS II and PS I needs switching from *state 2* to *state 1*, indicated by the concomitant growth of the fluorescence production (F<sub>m</sub>).

In this paper we studied the photochemical activity in *Mougeotia* filaments during state transitions under the effect of low temperature in chemically induced anaerobic conditions and in the presence of certain photosynthetic inhibitors.

## MATERIALS AND METHODS

The green alga *Mougeotia* sp. (AICB 560) is originated from the Culture Collection of Algae of the Institute of Biological Research, Cluj-Napoca (AICB)<sup>5</sup>. The alga was grown using Bold (BBM) nutritive solution under continuous air stirring and illumination with 485 μmol·m<sup>-2</sup>·s<sup>-1</sup>, at 22°C, during exponential growth.

### Treatment with light, low temperature and inhibitors

The inducement of the chlorophyll fluorescence with quenching analysis on short term was recorded in *state 1* and *2* by using light 1 and 2 after applying this working protocol:

- measurement light – 650 nm
- working manner: saturation pulse

- **V<sub>1</sub>**: actinic light excitation at 4112 μmol·m<sup>-2</sup>·s<sup>-1</sup> PAR (665 nm) for 10 seconds (for PS II excitation) – *state 2*

- **V<sub>2</sub>**: low temperature treatment (0<sup>0</sup>–2<sup>0</sup>C) for 30 minutes followed by actinic light excitation at 4112 μmol·m<sup>-2</sup>·s<sup>-1</sup> PAR (665 nm) for 10 seconds – *state 2*

- **V<sub>3</sub>**: low temperature treatment (0<sup>0</sup>–2<sup>0</sup>C) for 30 minutes in the presence of FCCP followed by actinic light excitation at 4112 μmol·m<sup>-2</sup>·s<sup>-1</sup> PAR (665 nm) for 10 seconds – *state 2*

- **V<sub>4</sub>**: low temperature treatment (0<sup>0</sup>–2<sup>0</sup>C) for 30 minutes in the presence of FCCP and iodoacetamide followed by actinic light excitation at 4112 μmol·m<sup>-2</sup>·s<sup>-1</sup> PAR (665 nm) for 10 seconds – *state 2*

- **V<sub>5</sub>**: low temperature treatment (0<sup>0</sup>–2<sup>0</sup>C) for 30 minutes in the presence of FCCP, DCMU and methylviologen followed by far-red light excitation (730 nm) for 10 seconds – *state 1*

- **V<sub>6</sub>**: low temperature treatment (0<sup>0</sup>–2<sup>0</sup>C) for 30 minutes in the presence of FCCP, DBMIB and methylviologen followed by far-red light excitation (730 nm) for 10 seconds – *state 1*.

### Chlorophyll fluorescence analysis

The chlorophyll fluorescence was measured using a PAM-100 fluorometer according to Schreiber *et al.*<sup>16</sup>. The fluorescence parameters and quenching analysis was performed by applying the saturation pulse method. The quantum yield of the photochemical energy conversion was calculated according to the equation Yield = ΔF/F<sub>m</sub> and the ratio F<sub>v</sub>/F<sub>m</sub> (F<sub>v</sub>/F<sub>m</sub> = F<sub>m</sub>–F<sub>0</sub>/F<sub>m</sub>) which gives information on the photochemical quantum yield of closed PS II reaction centers.

## RESULTS AND DISCUSSION

During fluorescence measurement at room temperature on algae whole cells, the fluorescence emission is reversed proportionally with the photochemical yield of PS II and is proportional with the light capture antenna's size. It is possible to see the changes in the antenna's size if the PS II's photochemistry is inhibited using DCMU<sup>8</sup>. The 650 nm light excites mainly the PS II photosystem inducing a increased sensitivity of PS I, and the far-red light excites the PS I, inducing a increased sensitivity of PS II<sup>23</sup>.

FCCP (carbonyl cyanide p-trifluoromethoxy-phenylhydrazone) is a decoupling agent which causes the transition to *state 2* by decreasing the ATP and the membrane potential. Iodoacetamide inhibits the activity of Rubisco enzyme (Calvin cycle), and DCMU [3-(3,4-dichlorofenil)-1,1-dimetilurea] inhibits the reduction of Q<sub>B</sub> from PS II so that the plastoquinone is oxidated by light.

The minimal fluorescence (F<sub>0</sub>) has decreased in *state 2* in the presence of preillumination with intense light associated to low temperature, more significantly in the anaerobiosis variants (V<sub>4</sub>) (Fig. 1). In *state 1*, favorable to the photochemical activity of PS I, in the presence of DCMU inhibitor which affects the linear electron transport and the methylviologen which is the artificial acceptor of

electrons from PS I, the  $F_0$  fluorescence has increased reaching the control value ( $V_5$ ). This proves that in the presence of methylviologen, DCMU inhibitor does not produce negative effects over the electron transport chain.  $F_0$  became almost equal to  $F_m$ . *State 2–state 1* transitions is stimulated by oxygen and electron acceptors of PS I such as methylviologen. The oxygen is an electron acceptor acting on the reducing side of PS I – the transfer of electrons to  $O_2$  is done by the Mehler reaction which is involved *state 2–state 1* transitions. The increase of  $F_m$  in *state 1* transition shows an increase of LHC II antenna. Methylviologen is an acceptor for the electrons from PS I being reoxidated through  $O_2$  and produces  $O_2^-$  which is used in the Mehler reactions. When the oxidation of  $Q_A$  does not take place, the electrons are blocked and the PS II does not pump electrons in the electron transport chain as in *state 2*, and  $F_0$  is equal to  $F_m$ . Methylviologen accelerates the electron transport towards  $O_2$ <sup>9</sup>.

The maximal fluorescence ( $F_m$ ) has decreased significantly both in *state 1* and *state 2*. It is notable the almost equal values of  $F_0$  and  $F_m$  in the *state 1* of photosystems ( $V_5$ ) (Fig. 1). The decline in the  $F_m$  has led to an decrease of the variable fluorescence ( $F_v$ ). The results are contradictory. Thus, methylviologen is used as a base for the linear electron transport and produces the  $F_m$  diminish, and DBMIB in the presence of methylviologen contributes to the  $F_m$  recovery<sup>11</sup>. In the preilluminated cells the inhibiting effect of DBMIB over the electron transport is increasing. DBMIB inhibits the kinase activation in the presence of light because reacts with cytochrome *b<sub>6</sub>f* and acts like a fluorescence quencher by binding to the  $Q_B$  place in PS II where DCMU also bonds. In *state 2* in dark, DBMIB does not inhibit the kinase activation – there is an increased level of LHC II phosphorylation, and when *state 2* has been reached under illumination, DBMIB inhibits the LHC II phosphorylation.

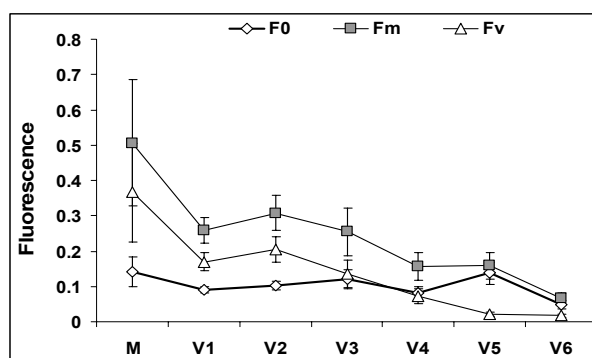


Fig. 1. Evolution of minimal, maximal and variable fluorescence in the conditions of photosystems' *state 1* and *2*. M= control;  $V_1$ – $V_6$  (see Material and Method).

The results obtained regarding the evolution of maximal and variable fluorescence emphasizes the appearance of photoinhibition in the electron transport chain which is induced by low temperature in association with intense light. Photochemical activity carries on in „down regulation” conditions.

The photochemical efficiency ( $F_v/F_m$ ) as well as the quantum yield of the photosynthetic electrons transport chain have decreased significantly in both redox states of the photosystems, highlighting the photoinhibition of the closed reaction centers (Fig. 2). In *state 1* in the presence of DBMIB inhibitor it was recorded a slight recovery of the photochemical activity, although DBMIB inhibits the linear electron flow<sup>13</sup>. This recovery is assigned to the cyclic electron current around PS I. The close numeric values for photochemical efficiency and quantum yield in all variants emphasized the lack of energized state in the thylakoidal membrane. In the state transitions, a decrease in the photochemical efficiency and quantum yield is due greatly to inhibitor effects of low temperature.

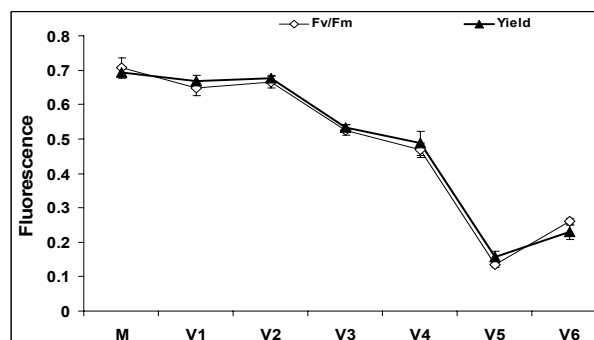


Fig. 2. Evolution of photochemical efficiency and quantum yield in redox *states 1* and *2*.

Low light exposure produces reversible decrease of photochemical efficiency because photoinhibition appears. The extent of the photoinhibition depends on the capacity of energy usage in photochemical reactions or in thermal dissipation, processes which are used for decreasing the excitation energy for the purpose of decreasing the photosystem's vulnerability to photoinhibition<sup>14</sup>. The lack of balance between synthesis and degradation of  $D_1$  protein produces an inhibition of the PS IIs' electrons transport during photoinhibition<sup>3</sup>.

The photochemical activity of photosystem II in *state 2* unreel under a decreased excitation pressure reported to the control sample (Fig. 3). In

*state 1* in the presence of DBIMB the excitation pressure has increased significantly, stating an increase in the proportion of closed (reduced)  $Q_A$  ( $V_6$ ). Functionally, a growth in the excitation pressure is translated in an increased proportion of primary acceptors  $Q_A$  in the reduced state, an increased concentration of zeaxanthin and a decreased antennary proteins LHC II. The state transitions are designated by the changes in the redox state of the plastoquinone pool, namely the reduced PQ leads to *state 2* as the core of oxidized PQ leads to *state 1*. The state transitions are accompanied by changes in the phosphorylation of LHC II which suggests that LHC II-kinase might be itself or be associated with a plastoquinol-binding protein. Two protein complexes display quinone/quinol-binding sites in the membrane-embedded photosynthetic electron transport chain: PS II and the cytochrome *b<sub>6</sub>f* complex<sup>23</sup>. The *state 1* favours the cyclic phosphorylation around PS I and cytochrome *b<sub>6</sub>f* helping the ATP production, and the *state 2* favours the lineary phosphorylation, producing NADPH for  $CO_2$  fixation.

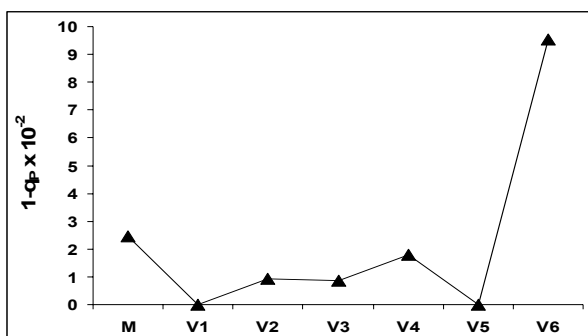


Fig. 3. Evolution of excitation pressure in photosystems' *state 1* and 2.

The state transitions are controlled by the need of ATP: the cells adapted to dark are in *state 2* when the ATP concentration is reduced, and the passing to *state 1* shows a restoration in the ATP content<sup>8</sup>. The light dependent electron transport from water to monodehydroascorbate is coupled to ATP formation with a ratio  $ATP/O_2 = 2$ <sup>10</sup>. The cyclic electron transport around PS I prevents photoinhibition in stress conditions by maintaining a low pH in the thylacoid lumen which allows the dissipation of the excitation energy excess<sup>12</sup>.

In conditions which reduce the light energy consumption in photochemistry and thermic dissipation, the chlorophyll fluorescence increases, while an increase in the excitation energy usage leads to fluorescence quenching<sup>14</sup>. The non-

radiative dissipation of excitation energy in the photosystems' antenna decreases  $F_0$  and  $F_m$  proportionally, and dissipation from reaction centers reduces the  $F_m$  only<sup>11</sup>. The fluorescence quenching associated to photoinhibition is not correlated directly to degradation of PS II reaction centers<sup>3</sup>.

The photochemical coefficient  $q_P$  has maintained at high values in *state 2*, overcoming the control value which states an increase in the proportion of open  $Q_A$  (Fig. 4). Functionally, the excitation energy has been effectively converted to photochemistry and has been correlated to the quenching of chlorophyll fluorescence. In *state 1*, in the presence of DCMU inhibitor,  $q_P$  has reached its maximum value, leading to a reach of the maximum level of photochemical energy conversion. Along with methylviologen, DCMU does not inhibit the electron flow between the two photosystems. In the presence of DBMIB, the linear transport of photosynthetic electrons has been inhibited ( $V_6$ ), leading to a decrease in the open  $Q_A$  proportion and to an increase in the non-photochemical dissipation of the excitation energy, respectively to an increase of the non-photochemical coefficient  $q_N$  which correlates to the excitation pressure (Fig. 4).

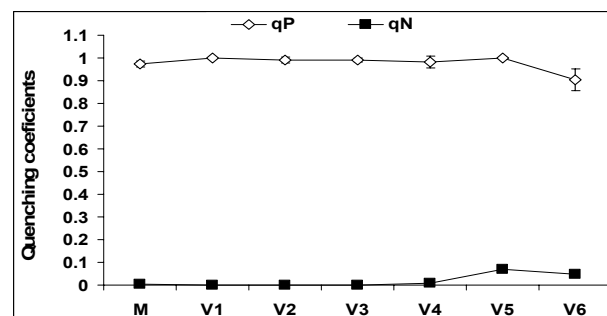


Fig. 4. Evolution of the photochemical and non-photochemical coefficients in photosystems' *state 1* and 2.

## CONCLUSIONS

1. The inducement of chlorophyll fluorescence with quenching analysis on a short period of time in *state 1* and 2 on algae whole cells marks out the proportionality ratio with the light harvesting antenna's size. The minimal fluorescence has decreased in *state 2* in the presence of preillumination with intense light and in association with low temperature or in anaerobiosis conditions, while in *state 1* in the presence of DCMU and methylviologen  $F_0$  has equaled the control values. In the presence of methylviologen, DCMU does not inhibit the electron transport

chain, and  $F_0$  has become almost equal to  $F_m$ . The maximal fluorescence has decreased in both *state 1* and *2* which lead to a decrease of the variable fluorescence. The photochemical efficiency as well as the quantum yield were reduced in both redox states, emphasizing the inhibition of the closed reaction centers.

2. The photochemical activity in *state 2* carried on under a low excitation pressure, and in *state 1* in the presence of DBIMB the excitation pressure increased which states a growth in the closed (reduced)  $Q_A$  proportion, respectively, the decrease of LHC II antenna proteins. *State 1* favours cyclic phosphorylation around PS I and cytochrome *b<sub>6</sub>f* helping the ATP production, and *state 2* favours linear phosphorylation, producing NADPH for CO<sub>2</sub> fixation. The photochemical coefficient values have increased in *state 2* which states an increase of the opened  $Q_A$  proportion. Functionally, the excitation energy was effectively converted to photochemistry and was correlated with a decrease in the chlorophyll fluorescence. In the presence of DBMIB, the linear transport of photosynthetic electrons was inhibited, leading to a decrease of the opened  $Q_A$  proportion and to an increase of the non-photochemical dissipation of the excitation energy, respectively to a growth of the non-photochemical coefficient which is correlated to the excitation energy.

3. The results obtained reveal the photoinhibition state at the level of the electron transport chain which is induced by low temperature in association with intense light. The photochemical activity unreeles in down regulation conditions. The Mehler reaction and the cyclic electron current around PS I can contribute to the increase of ATP/NADPH ratio. Maintaining the electron flow in thylakoids in the presence of an enough quantity of NADP<sup>+</sup> as an electron acceptor is essential to the protection of chloroplasts against photooxidative stress, process in which participate the cyclic electron current and the water-water cycle around PS I.

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