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THE EFFECT OF DIFFERENT LIGHT INTENSITIES ON PHOTOCHEMICAL ACTIVITY IN *MICROCYSTIS AERUGINOSA* AICB 702 STRAIN (CYANOPHYTA)

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SUMMARY. *Microcystis aeruginosa* AICB 702 was grown at room temperature, on GZ medium, in air-lift conditions, using a medium light intensity of 260 μmol. $m^{-2}.s^{-1}$. The exponential-phase cells were exposed to 800, 1500 and 2100 μmol. $m^{-2}.s^{-1}$ light intensities and the fluorescence measurements were performed after 15, 30, 45, 60, 75, 90, 105 and 120 minutes of light exposure. Dark-adapted probes were analyzed after 120 minutes recovery period. The oxidation state of primary acceptor and the fraction of the reaction centers are reduced (damaged), the photochemical process and the light harvesting are slightly inhibited when an 800 μmol. $m^{-2}.s^{-1}$ light intensity is used. Also, the reduction of the primary acceptor and the reaction centers closure were maximal. The F_v/F_m and Y(II) quantum yield were inhibited. The Fv/Fm values registered toward the end of the experiment represented 46.5% of the theoretical value, which also indicates the ratio of the PS II reaction centers that were photoinhibited. The non-regulated energy dissipation increased, and the high values of qP and qL coefficients showed an enhanced photochemical process with a low fluorescence emission. Increased F_0 and F_m and Fv decreased when 1500 μmol.$m^{-2}.s^{-1}$ was used. The Fv/Fm and Y(II) diminished and the non-regulated energy dissipation increased. When a light intensity of 2100 μmol.$m^{-2}.s^{-1}$was used, the Fo raised during a 90 min period, then decreased to 85%. Fm and Fv decreased, also. The Fv/Fm and Y(II) diminished, while the non-regulated energy dissipation increased. The decrease of F_0, F_m and Fv/Fm yield and that of the Y(II) certified the photoinhibition effect of 2100 μmol.$m^{-2}.s^{-1}$ light intensity on the activities carried out by the photosystems antenna. When an 800 μmol.$m^{-2}.s^{-1}$ light intensity was used the recovery of the photosynthetic activity was faster compared with other high-light intensities treatments.

Keywords: chlorophyll fluorescence, maximal fluorescence Fm, minimal fluorescence F0, maximal PS II quantum yield (Fv/Fm), effective PS II quantum yield (Y_{II}), quantum yield of non-regulated energy dissipation (Y_{NO}), recovery, photoinhibition, coefficient of photochemical quenching (qP, qL).

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Introduction

Cyanobacteria showed different strategies of acclimation and adaptation to light. Numerous cyanobacterial strains possess the ability to change their phycobilisomes composition in response to different wavelengths of light (Everroad *et al*., 2006). This ability, known as *chromatic adaptation* is present under various forms in different species (Kehoe and Guțu, 2006). The cyanobacterial sensory proteins initiate a signal transduction cascade in response to environmental signal, consisting in recognizing and translating the signal, followed by the cell response (Montgomery, 2007). The photoinhibition term describes the reduction of the photosynthetic capacity which is induced by the exposure to visible light (400-700 nm), regardless of the changes in pigments concentrations (Powles, 1984).

In Cyanobacteria, the photosynthetic system is related with the main metabolic pathways, thus the signal of the chlorophyll fluorescence can provide information on photosynthesis and the acclimation state. According to Campbell (1998), the excitation energy can be used in four distinct pathways as it follows:

- the excited electron produced in photochemical reactions which are carried out by the chlorophyll reaction centers is transferred to the electron transport chain.
- the high-energy electron falls back into its ground state and releases its energy as thermal energy dissipation.
- the transfer of the excitation energy to the adjacent pigment by the light-harvesting antenna system.
- the fluorescence is emitted with a lower energy, but a longer wavelength than the absorbed photon.

Although, these processes are in competition with each other, the energy of an excited molecule is used in the pathway that has the highest constant rate. The chlorophyll fluorescence yield is usually low and, *in vivo,* the chlorophyll fluorescence is produced by the PS II. The PS I contribution to the fluorescence value, which is presumed to have a constant level, is 15 to 20% (Roelofs *et al.,* 1992; Trissl *et al.,* 1993).

The photosynthetic organisms have developed multiple protection mechanisms to survive in high-light conditions. Recently, a possible mechanism dissipation of the excess energy absorbed by the phycobilisomes of the extramembrane antenna has been described (Wilson *et al.,* 2006). This mechanism which occurs in phycobilisomes is characterized by the fluorescence quenching under blue light and a carotenoid protein – *orange carotenoid protein* (OCP), encoded by the *srl1963* gene is especially involved in this process, also (Wilson *et al.,* 2006).

This study shows the results of the photochemical activity of PS II photosystem in *Microcystis aeruginosa* AICB 702 cyanobacterium (Chroococcales) under various light intensities.

Material and methods

The cyanobacterium *Microcystis aeruginosa* AICB 702 was cultivated for eleven days on GZ medium in conditions of 260 μmol.m^{-2}.s^{-1} light intensity, at room temperature and in air-lift conditions. The cyanobacterial cells reaching the exponential phase of growth were exposed to high-light intensity treatments of 800, 1500, and 2100 μmol.m-2.s-1 respectively. An incandescent light source (FHI-5000 W) was used and the light intensity measurements were taken with Quantum Sensor QSPAR Hansatech. The effect of light treatment was monitored at 15, 30, 45, 60, 75, 90, 105 and 120 min after the initiation of the exposure. The chlorophyll fluorescence parameters were taken with Walz Dual-100 fluorometer on light exposed probes. After 120 min recovery period when the light exposure was ceased, fluorescence parameters were measured on dark-adapted probes.

The assimilatory pigments (chlorophyll *a,* carotenoids) were extracted in acetone and quantified by spectrophotometric method based on their absorption coefficients (Arnon, 1949; Lichtenthaler and Wellburn, 1983), and their identification was made based on their absorption peak obtained with Jasco V-630 spectrophotometer. Phycobiliproteins were estimated based on Gantt and Lipschultz (1974). The results were expressed as mg/L of cellular suspension.

Results and discussions

The biomass of *Microcystis aeruginosa* AICB 702 strain showed an optical density (OD_{680}) of 0.518. The *in vivo* absorption spectra emphasized the spectral features of the carotenoids in the blue range (448 nm), of the chlorophyll *a* at 436 nm and 680 nm and that of the phycobiliproteins at 621 nm (fig. 1). Phycobilisomes are mainly composed of phycobiliproteins and they are attached to the surface of the thylakoid external membrane. Phycobilisomes function as peripheral light harvesting organelles (Lemasson *et al.,* 1973).

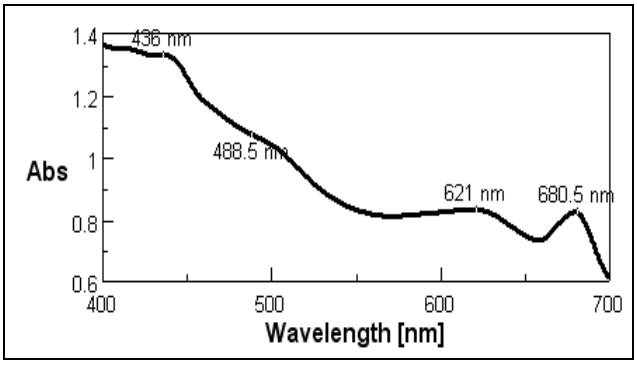

Fig. 1. *In vivo* absorption spectrum of *Microcystis aeruginosa* AICB 702 cell suspension cultivated in normal growth conditions.

The content in assimilatory pigments measured at the starting point of the experiment, prior of exposing the culture to high-light conditions, is listed in Table 1.

Table 1

The quantity of assimilatory pigments at *Microcystis aeruginosa* AICB 702 strain mg/L.

Cyanobacterial strain	Chlorophyll *a*	Carotenoids	*a*/c	Phycobiliproteins		
				Phycocyanin	Allophyco-cyanin	C-phyco-erythrin
Microcystis aeruginosa AICB 702	5.646	1.342	4.20	4.165	4.642	3.450

When the environmental light in changing, the stoichiometric regulation of the photosystems (PS I/PS II ratio) allow an increasing in the efficiency of the photosynthetic electron transport chain, due to the different absorption spectra of the antenna (Sonoike *et al.,* 2001). The content of PS I was decreasing relative to PS II when the cells were exposed to the light which is mostly absorbed by PS I and it increased when the light was mostly absorbed by PS II (Melis *et al.,* 1989; Cunningham *et al.,* 1990).

The evolution of the chlorophyll fluorescence parameters at *Microcystis aeruginosa* AICB 702 exposed to an 800 $\mu mol.m^{-2}.s^{-1}$ light intensity is shown in fig. 2. Generally, the minimal fluorescence, $F_{0,}$ increased up to 110%, relative to control sample. This fact shows that the oxidation of the primary acceptor and the opening of the reaction centers were slowed down (disturbed) and the photochemical process and light energy harvesting were inhibited. The maximal fluorescence F_m increased gradually. By the increasing of fluorescence from F_0 minimal level to F_m maximum level, the PS II primary electron acceptor became totally reduced, the photochemistry was blocked and the reaction centers closed. F_m increasing pointed out that the reduction of the primary electron acceptor and the closure of the reaction centers were maximal. The variable fluorescence reached negative values relative to control probe, except for the first 30 minutes of exposure.

The maximal quantum yield ($F_v/F_{m)}$ and the effective quantum yield Y(II) were inhibited gradually, and decreased by 90% at the end of the experiment (fig. 2B). Fv/Fm value recorded at the end of the experiment was 0.382, which represents 46.5% from the theoretical value. The Fv/Fm theoretical value of 0.82 outlined the maximum fraction of absorbed photons which are used in photochemistry. The values lower than 0.8 indicates the ratio of the PS II reaction centers that are photoinhibited.

The quantum yield of non-regulated energy dissipation, Y(NO), increased up to 106%. In the first 15 minutes of high-light exposure the dissipation lowered below the control sample value. In theory, the quantum yield of non-regulated energy dissipation conveys the sum of the energy dissipation processes which occur at the

level of photosystems antenna. qP and qL photochemical coefficients were stabilized at maximum values (fig. 2 C). The recorded high values confirmed the maximal opening of the reaction centers ratio. The qP coefficient enables the estimation of the fraction of oxidized quinone acceptor from PS II and that of the open PS II centers (Grace and Logan, 1996). The high values of qP and qL coefficients pointed out an enhance photochemical process with a low fluorescence emission.

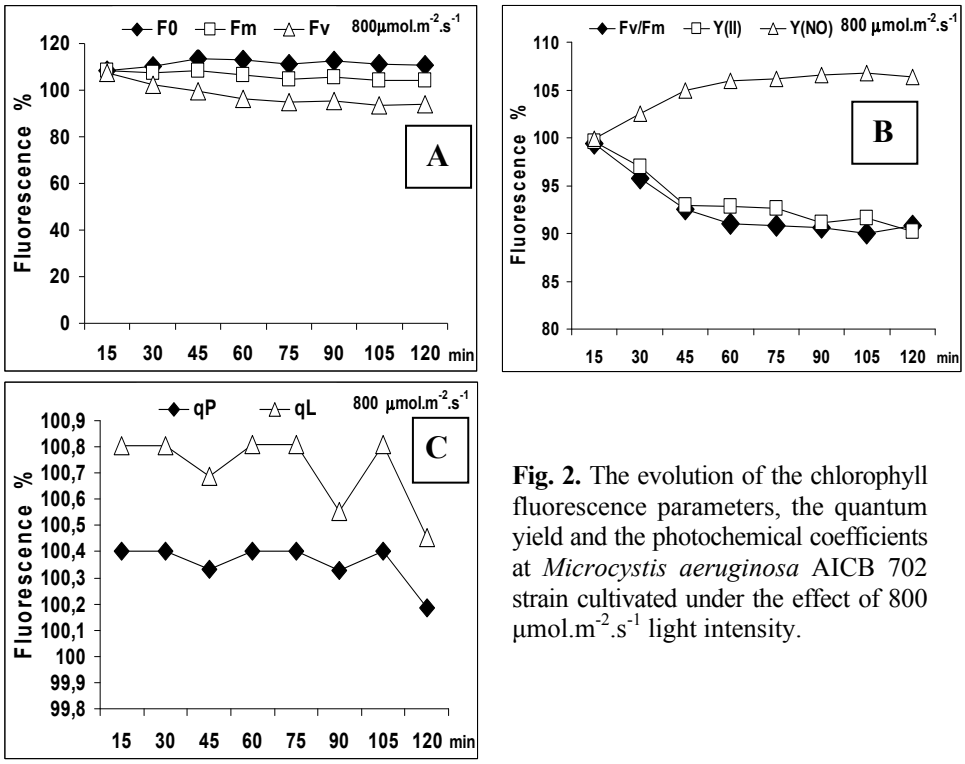

Fig. 2. The evolution of the chlorophyll fluorescence parameters, the quantum yield and the photochemical coefficients at *Microcystis aeruginosa* AICB 702 strain cultivated under the effect of 800 $\mu mol.m^{-2}.s^{-1}$ light intensity.

The fluorescence photochemical quenching is maximal and the fluorescence yield is low, as the PS II reaction centers are open and the photochemistry potential is maximal. The photochemical quenching is null and the fluorescence yield is maximal, while the PS II reaction centers are closed and photochemical processes are ceased. The excitation energy is transferred from the closed to the open reaction centers through the oxidized plastoquinone.

When exposed to high-light conditions, the capacity of cyanobacterial strains to maintain the PS II reaction centers in an open state pointed out a complex and flexible electron transport system (Geerts *et al.,* 1994; Mi *et al.,* 1995; Schubert *et al.,* 1995; Shyam *et al.,* 1993) and a high PS I/PS II ratio, also (Campbell *et al*., 1996; Fujita *et al.,* 1985; Papageorgiou, 1996).

The evolution of chlorophyll fluorescence parameters when exposed to 1500 μmol.m^{-2}.s^{-1} light intensity is shown in fig. 3. F_0 increased up to 114%. Generally, F_m parameter lowered to 87%. Interestingly, F_m value increased in the first 15 minutes of light exposure. The variable fluorescence decreased proportionally to 49%. The oxidation and the reduction of the primary acceptor Q_A and the opening and the closure of the reaction centers were disturbed.

The maximal quantum yield F_v/F_m and the effective quantum yield Y(II) decreased gradually to 57% (fig. 3 B). The quantum yield of non-regulated energy dissipation Y(NO), increased proportionally up to 130%. Generally, the coefficients of the photochemical quenching, qP and qL, recorded maximal values, emphasizing the oxidized state of the primary acceptor Q_A (fig. 3 C).

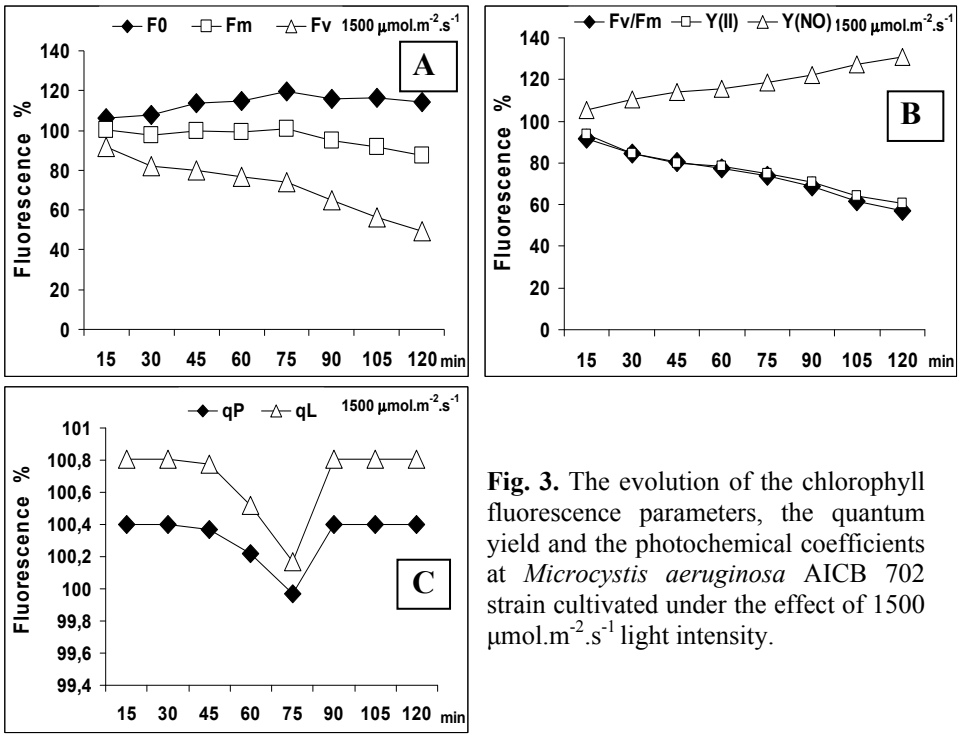

Fig. 3. The evolution of the chlorophyll fluorescence parameters, the quantum yield and the photochemical coefficients at *Microcystis aeruginosa* AICB 702 strain cultivated under the effect of 1500 μmol.m^{-2}.s^{-1} light intensity.

The chlorophyll fluorescence induction kinetics suffered some changes when the cell suspension was exposed to the 2100 μmol.m^{-2}.s^{-1} light intensity (fig. 4). Thus, F_0 registered high values for 90 minutes, than decreased to 85%. After the first 15 minutes, Fm dropped to 59%. The decrease of the maximal fluorescence caused a dropping in the variable fluorescence to 24%.

The maximal quantum yield F_v/F_m and the effective quantum yield Y(II) decreased proportionally to 41%, pointing out the rising of the fraction of photoinhibited reaction centers (fig. 4 B). The quantum yield of the non-regulated energy dissipation Y(NO) increased up to 142%.

The coefficients of photochemical quenching, qP and qL kept high values, which certify the high ratio of the opened reaction centers (fig. 4 C). The high values of the coefficients pointed out the usage of the excitation energy in the photochemistry process in the reaction centers, followed by chlorophyll fluorescence decay.

The initiation of the photoinhibition process occurs at the level of the photosystems antenna, when exposing to 2100 μmol.m^{-2}.s^{-1}, as the F_0, F_m fluorescence parameters decayed and the quantum yields Fv/Fm and Y(II) decreased.

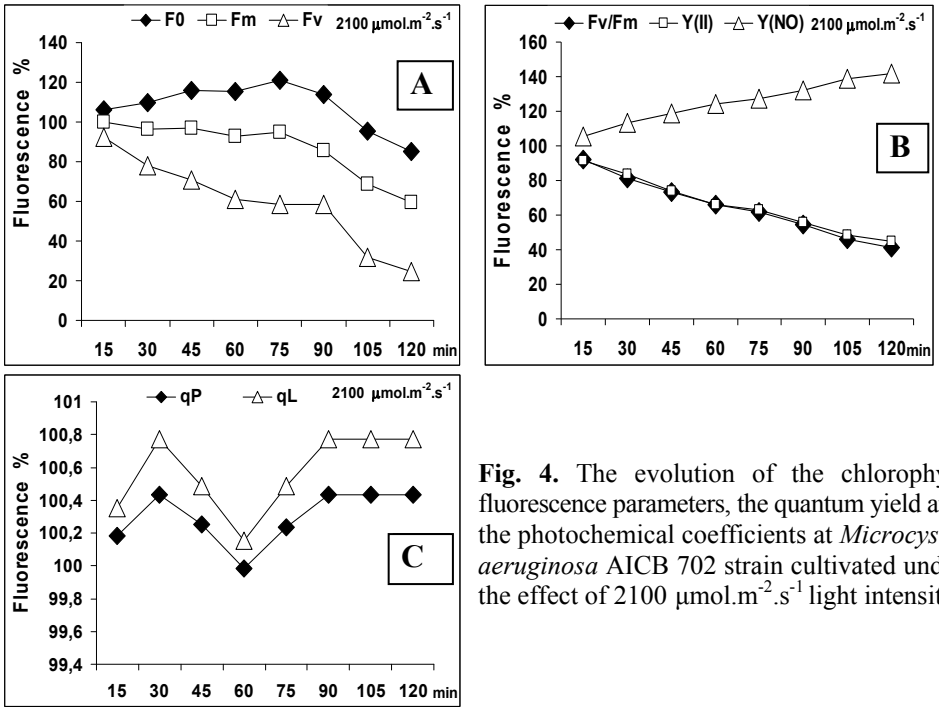

Fig. 4. The evolution of the chlorophyll fluorescence parameters, the quantum yield and the photochemical coefficients at *Microcystis aeruginosa* AICB 702 strain cultivated under the effect of 2100 μmol.m^{-2}.s^{-1} light intensity.

After 120 minutes recovery period of the dark-adapted samples, F_0 reached values close to the control probe value, more significantly in the case of 800 and 1500 μmol.m^{-2}.s^{-1} variants (fig. 5). Fm parameter kept a low value which induced a reduction in the value of the variable fluorescence. The maximal quantum yield and the PS II effective quantum yield remained low, except for the 800 μmol.m^{-2}.s^{-1} variant, when the recovery was faster. The quantum yield of the non-regulated energy dissipation Y(NO) kept high value. When exposing to an 800 μmol.m^{-2}.s^{-1} light intensity, the recovery of the photosynthetic activity was faster compared to the other used high light intensities.

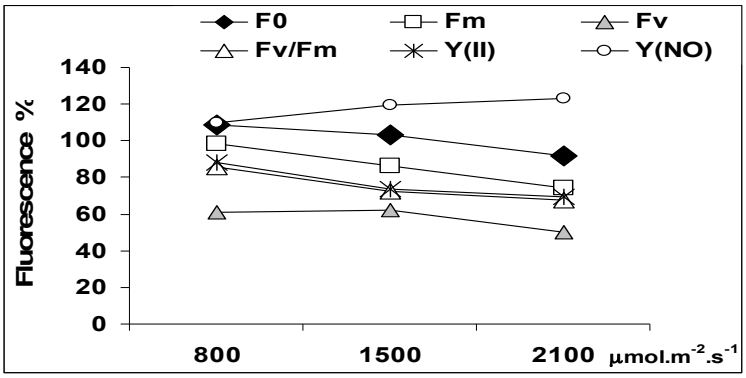

Fig. 5. The evolution of the chlorophyll fluorescence parameters at *Microcystis aeruginosa* AICB 702, in the recovery period that followed the high-light exposure **treatment.**

During the recovery period, the coefficients of the photochemical quenching, qP and qL reached values close to one, which confirm the maximal opening state of the PS II reaction centers (fig. 6).

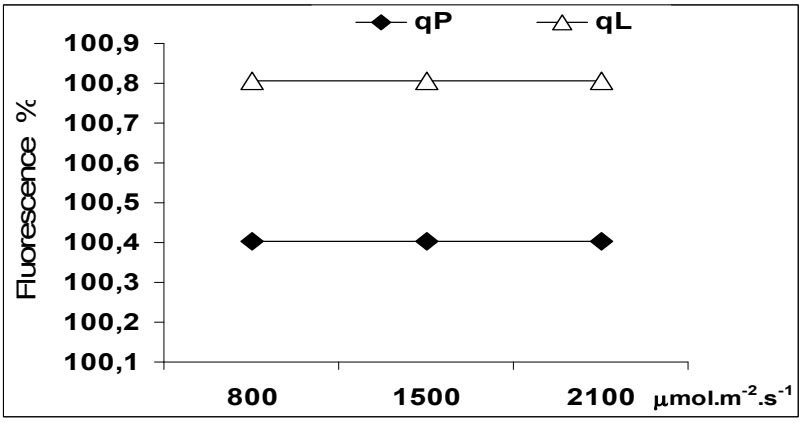

Fig. 6. The evolution of the photochemical coefficients at *Microcystis aeruginosa* AICB 702, in the recovery period that followed the high-light exposure treatment.

Conclusions

Microcystis aeruginosa AICB 702 cells were grown on GZ medium, at room temperature, under air-lift conditions, using a light intensity of 260 µmol.m^{-2}.s^{-1}. The components of the photosynthetic apparatus such as chlorophyll *a,* various types of carotenoids and phycobiliproteins form the light harvesting antenna. The pigment quantity and other components of the photosynthetic apparatus are regulated in response to the light intensity conditions.

The oxidation state of the primary electron acceptor and the opening level of the reaction centers are reduced (deranged), the photochemical process and the light harvesting are slightly inhibited when an 800 μmol. $m^{-2}.s^{-1}$ light intensity is used. Also, the reduction of the primary acceptor and the reaction centers closure were maximal. The maximal PS II quantum yield ($F_v/F_{m)}$ and the effective quantum yield Y(II) were inhibited. The Fv/Fm values registered toward the end of the experiment represented 46.5% of the theoretical value, which also indicates the ratio of the PS II reaction centers that were photoinhibited. The quantum yield of the non-regulated energy dissipation Y(NO) increased at the level of the photosystems antenna. The high values of qP and qL coefficients showed an enhance photochemical process with a low fluorescence emission.

The F_0 parameter increased and F_m and Fv decreased when 1500 $\mu mol.m^{-2}.s^{-1}$ was used. The oxidation and the reduction of the primary electron acceptor Q_A and the opening and the closure of the reaction centers were disturbed. The maximal quantum yield and the effective quantum yield Y(II) diminished and the quantum yield of non-regulated energy dissipation increased. Generally, the photochemical coefficients qP and qL reached higher values relative to control, fact that characterizes the opening state of the reaction centers and the oxidized state of the primary electron acceptor $Q_{A,}$ respectively.

When a light intensity of 2100 $\mu mol.m^{-2}.s^{-1}$was used, the Fo parameter raised during a 90 min period, then decreased to 85%. Fm and Fv decreased. The maximal quantum yield and the effective quantum yield Y(II) decreased, while the quantum yield on non-regulated energy dissipation Y(NO) increased. The photochemical coefficients qP and qL were higher relative to control, which assert the high ratio of the open reaction centers. The decreasing in $F_{0,}$ F_m fluorescence parameters, Fv/Fm yield and that of the effective quantum yield Y(II) certified the photoinhibition effect of 2100 $\mu mol.m^{-2}.s^{-1}$ light intensity over the activities carried out by the photosystems antenna.

During the recovery period, F_0 reached values close to the control, in the case of 800 and 1500 $\mu mol.m^{-2}.s^{-1}$ light intensity treatments. Fm parameter was diminised. The maximal quantum yield and the effective quantum yield remained low, except for the 800 $\mu mol.m^{-2}.s^{-1}$ variant, when the recovery was faster. The quantum yield of the non-regulated energy dissipation was characterized by a high value. When exposing to an 800 $\mu mol.m^{-2}.s^{-1}$ light intensity, the recovery of the photosynthetic activity was faster compared to the other used high-light intensities.

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