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THE RELATION BETWEEN THE WATER-WATER CYCLE AND THE STATE TRANSITIONS IN THE GREEN ALGA *MOUGEOTIA*, STRAIN AICB 560

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Abstract: The activity of the antioxidative enzymes during the state transitions in the presence of specific inhibitors in the algal suspensions *Mougeotia* was evaluated. The state transitions were obtained by excitation with light *1* (over 700 nm) (*state1*), and light *2* (620 nm) under aerobic conditions and in the presence of FCCP (*state 2*). The superoxide-dismutase activity has been significantly enhanced in *state 1* in the presence of DCMU which causes the use of electron in PS I to deactivate the oxygen reactive species. By blocking the PS II, the cyclic electron flow around PS I operates at maximum photosynthesis rate. In *state 1* in the presence of DCMU, the ascorbate peroxidase intensified its activity due to the PS I activity activation by light *1*. In *state 2* the ascorbate peroxidase operates at higher intensities. The activity of monodehydroascorbate reductase has also been intensified during the state transitions. The state transitions stimulated the glutathione reductase and NADPH dehydrogenase activity in *state 1* in the presence of DCMU and in *state 2* in the presence of chloramphenicol. Under the action of FCCP, light *2* accelerated the $NADP^+$ photoreduction by stimulating the NADPH dehydrogenase activity. The lipids peroxidation has been significantly decreased, while in the presence of DCMU this activity has been doubled. The induced activation of the antioxidative enzymes during the state transitions certifies that the relative flux of the O_2 reducing equivalents by the Mehler reaction is high, and the presence of $NADP^+$ as electron acceptor is essential, to this process contributing the cyclic electron flow and the water-water cycle around PS I.

Keywords: antioxidant, ascorbate peroxidase, monodehydroascorbate reductase, cyclic electron transfer, glutathione reductase, photooxidative stress, superoxide dismutase, superoxide anion, scanvenging of reactive oxygen species

Introduction

Plants use various mechanisms for the protection of the photosynthetic apparatus against the destructive effects of the excess light absorption. The high light causes an enhancement of the electron flow rate from the O_2 molecule through the Mehler reaction. Such a non-assimilator flow may drain the electron excess through the electron carrier chain or it implies an adjusting role by enhancing the trans-thylakoidal gradient [22, 29].

The O_2 reduction in chloroplast is inevitable and it leads to the superoxide anion radicals (O_2^-) and H_2O_2. In normal chloroplasts, the O_2 photoreduction is estimated to be about 5-27% of the total electron flow [3]. The superoxide anion, H_2O_2, the hydroxyl radicals (OH), and the singlet oxygen ($^1O_2$) are very toxic, producing a destructive effect against the function of the chloroplasts structure. The protection against toxicity of the reactive oxygen species (ROS) is conferred by an enzymatic and non-enzymatic antioxidant system that is concentrated into the chloroplasts [2, 30].

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The PS I and PS II reaction centers are the main generation places for the oxygen reactive species. The oxygen photoreduction to the hydrogen peroxide was discovered by Mehler [18]. The chloroplasts reduces H_2O_2 by ascorbate peroxidase (APX) by using the electron derived from water in the PS I like this:

1. $2H_2O \Rightarrow 4e^- + O_2 + 4H^+$ (PS II)
2. $2O_2 + 2e^- \Rightarrow 2O_2^-$ (PS I)
3. $2O_2^- + 2H^+ \Rightarrow H_2O_2 + O_2$ (superoxide dismutase)
4. $H_2O_2 + 2AsA \Rightarrow 2H_2O + 2MDA$ (ascorbate peroxidase)
5. $2MDA + 2Fd_{red} \Rightarrow 2AsA + 2Fd$ (spontaneous)
6. $2MDA + NADPH \Rightarrow 2AsA + NADP^+$ (MDA reductase)
7. $2MDA \Rightarrow AsA + DHA$
   $DHA + 2GSH \Rightarrow AsA + GSSG$ (DHA reductase)
8. $GSSG + NADPH \Rightarrow 2GSH + NADP^+$ (glutathione reductase)
   $2Fd \quad ori \quad NADP^+ + 2e^- \Rightarrow 2Fd_{red} \quad ori \quad NADPH$ (PS I)

The first reduced product was identified as superoxide anion (O_2^-) and its disproportionated products: H_2O_2 and O_2 by superoxide-dismutase (SOD) catalysis. H_2O_2 is reduced to water by ascorbate (AsA), then is catalyzed by APX and the ascorbate is oxidized to monodehydroascorbate (MDA). Subsequently the MDH is reduced directly to ascorbate by reduced ferredoxin, or by NADPH, catalyzed by the monodehydroascorbate reductase (MDAR). If MDA is not directly reduced to ascorbate, it is suddenly disproportionated to dehydroascorbate (DHA). DHA is then reduced to ascorbate by reduced glutathione (GSH) which is catalyzed by dehydroascorbate reductase (DHAR). Eventually, ferredoxin and $NADP^+$ are reduced to PS I in order to regenerate the ascorbate. Thus, in any mode for regenerating the ascorbate, half of the electron derived from water in PS II are used in order to reduce the univalent oxygen reduction obtaining water, and other half for the generation of the reductants in the reduction of H_2O_2, this process being known as “the water-water cycle” [1].

In this study the activity of the antioxidative enzymes during the state transitions was evaluated in the presence of certain specific inhibitors in the algal suspensions *Mougeotia*.

Materials and Methods

The green alga *Mougeotia sp.* Agardh (AICB 560), derives from the Culture Collection of Algae of the Institute of Biological Research, Cluj-Napoca (AICB) [4].

Growth conditions.

The strain AICB 560 was grown for 23 days in Bold (BBM) nutritive solution at 22^0C under continuous air stirring and illumination with 630 $\mu mol.m^{-2}.s^{-1}$.

Treatment with light and inhibitors.

The 5000 $\mu mol.m^{-2}.s^{-1}$ PAR light intensity (photosynthetic active radiation) was applied for 60 minutes, at room temperature in the presence of certain specific inhibitors: 300 µM DCMU 3(3,4-dichlorophenyl)-1,1-dimethylurea), 20 mM DBMIB (2,5-dibromo-3 methyl-6-isopropyl-p-benzochinone), 230 µM chloramphenicol and 2 µM FCCP (carbonylcyanide-p(trifluoromethoxy)- phenylhydrazone). 5000 W halogen lamps were used as light source.

The state transitions were reached by photosystems excitation with different lights: *state 1*: light *1* (over 700 nm), far-red, emitted by combining blue and red filters at 470 $\mu mol.m^{-2}.s^{-1}$ light intensity, which activates PS I; *state 2*: light *2* (620 nm) specific for PS II, emitted by a red

filter with 2300 µmol.m^{-2}.s^{-1} light intensity. The emission intensities were achieved by passing the 5000 µmol.m^{-2}.s^{-1} light through the combination of these filters.

Enzymes analyses.

The antioxidative enzymes activity was determined at room temperature. The extraction medium contains 50 mM potassium phosphate buffer, pH = 7.8; 1 mM EDTA; 10 mM mercaptoethanol and 2% PVP (polyvinylpyrrolidone).

The *ascorbate peroxidase* (APX) was determined according to Leipner [16]. In the extraction medium 1 mM sodium ascorbate was added. The extraction medium contains: 80 mM potassium phosphate buffer pH = 7.0; 200 µM DTPA, 1 mM ascorbate and 250 µM H_2O_2 to which the enzymatic extract was added. The enzyme activity was determined by measuring the absorption at 290 nm using the extinction coefficient ε = 2,8 $mM^{-1}.cm^{-1}$.

Monodehydroascorbate reductase (MDAR) was determined by modifying the Leipner method [16]. The reaction medium contains: 80 mM potassium phosphate buffer pH = 7.8; 200 µM DTPA; 1 mM ascorbate; 0,12 mM NADPH, 1 ascorbate oxidase unit and enzymatic extract. The NADPH oxidation was evaluated by measuring the absorption at 340 nm. The enzyme activity was analyzed by using the extinction coefficient ε = 6,2 $mM^{-1}.cm^{-1}$.

Superoxide dismutase (SOD) was evaluated by modifying the Leipner method [16]. The reaction medium contains: 80 mM potassium phosphate buffer pH = 7.8; 200 µM DTPA; 13 mM methionine, 1mM NBT (nitroblue tetrazolium), 4 mM riboflavin. After illumination the enzymatic extract was added and the absorption at 560 nm was measured. A SOD unit represents the quantity of enzymatic extract that causes the absorption decrease with 50%.

Glutathione reductase (GR) was measured by modifying the Leipner method [16]. The reaction medium contains: 80 mM potassium phosphate buffer pH = 7.8; 200 µM DTPA; 1 mM oxidized glutathione (GSSG) and 0.12 mM NADPH. The enzyme activity was estimated by measuring the absorption at 340 nm using the extinction coefficient ε = 6.2 $mM^{-1}.cm^{-1}$.

NAD(P)H-dehydrogenase determination. The reaction medium contains the enzymatic extract in Tris-HCl buffer, 0.12 mM NAD(P)H and 120 µM menadione. The NAD(P)H-dehydrogenase activity in dark was spectrophotometrically analyzed by measuring the absorption at 340 nm. The enzyme activity was expressed in µmol/minute/mg chlorophyll a using the extinction coefficient ε = 6.23 $mM^{-1}.cm^{-1}$.

Lipids peroxidation was estimated according to Venisse et al. [30]. The algal material was extracted with trichloroacetic acid 10%. The extraction medium containing vegetal extract, trichloroacetic acid 10% and thiobarbituric acid 0.2%, was boiled for 30 minutes at 90°C, and then it was clarified through centrifugation. The TBARS content (reactive species of thiobarbituric acid) was measured on the basis of the absorption at 532 nm, using the extinction coefficient ε = 155 $mM^{-1}.cm^{-1}$.

Results and Discussions

The photoproduction rate of the reactive oxygen species enhances when the photons are more than necessary for the CO_2 assimilation. In this case, the relaxation systems acts on the photoproduction of oxygen reactive species at the level of chloroplasts, such is the photorespiration, the cyclic electron flow through PS I or PS II and the down regulation of PS II quantic yield, regulates through the xanthophyll cycle and the trans-thylakoidal protonic gradient [2]. The unfavorable environmental conditions cause the enhancement of the O_2 photosynthetic electron flux, this leading to an increased production of superoxide, H_2O_2 and hydroxyl radicals that limit the $NADP^+$ concentration during the electron acceptation from PS I [1].

The oxygen reactive species such are the superoxide anion radical, the hydrogen peroxide, the hydroxyl radical and the singlet oxygen are toxic for the cell, so the fast removal of the active oxygen is essential for the protection of cells against the oxidative injuries [26]. These reactive O_2 species are removed from the chloroplasts by a group of enzymes: SOD, GR, DHAR,

MDHAR and APX (catalase lacks into the chloroplasts) [1]. The increased concentration of these enzymes may limit the photodamage [12]. In normal conditions, the best part of the reductants that were generated through the electron transport are used during the CO_2 assimilation, and other pathways, like the nitrogen metabolism, O_2 reduction during the photorespiration and the Mehler reaction, are maintained at minimum values [5].

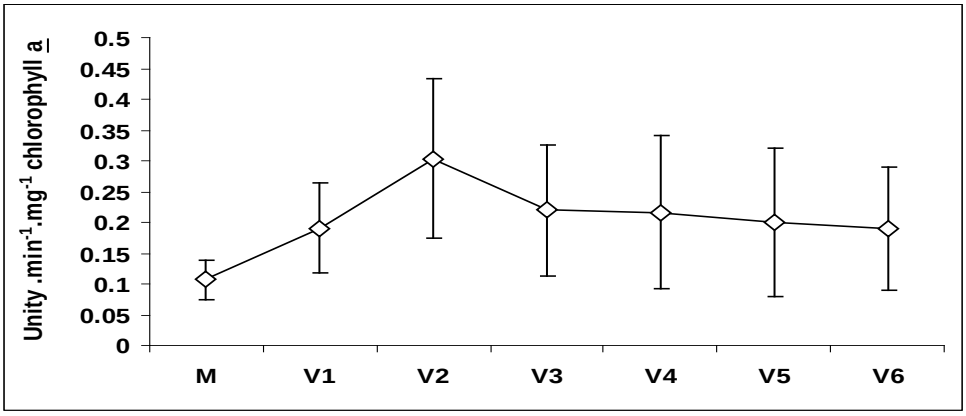

Fig. 1: The activity of superoxide dismutase (SOD): M = control; *State 1*>V_1=light 1; V_2 – light 1 + DCMU; *State 2* > V_3 – light 2 + FCCP; V_4 – light 2 + FCCP + DCMU; V_5 – light 2 + FCCP + DBMIB; V_6 – light 2 + FCCP + chloramphenicol

In photosynthetic organisms the superoxide is produced through the univalent photoreduction of dioxygen in PS I in the thylakoids when the photosynthesis is saturated by light [19, 29]. The superoxide dismutase (SOD) catalyses the dismutation of the O_2^- superoxide radicals producing molecular oxygen and hydrogen peroxide (H_2O_2), leading to the decrease of the O_2^- concentration, this being the first step in the enzymatic system for the removal of the active oxygen. The reaction takes place spontaneous and very fast in aqueous solution, and the stability of the superoxide anion radical in enhanced concomitantly with the increase of pH [17]. In plants there are three SOD isoforms: CuZn-SOD – active in cytosol and in the stroma; Fe-SOD – active in chloroplasts stroma and Mn-SOD – active in mitochondria [15]. Five SOD isozymes have been described: Cu/Zn SOD-1 inside of the chloroplasts and etioplasts, MnSOD-3 in mitochondria and three Cu/Zn SOD-2, Cu/Zn SOD-4 and Cu/Zn SOD-5 isoenzymes located in the cytosol [9, 27].

Comparatively to the control values, the SOD activity has enhanced in both photochemical states, more significantly in *state 1* in the presence of DCMU, which causes the use of electron in PS I toward the deactivation of the oxygen reactive species, by blocking the photosynthetic electron transport from PS II. Blocking PS II, the cyclic electron flow around PS I operates at maximum photosynthesis rate inducing the ATP synthesis, here being also involved the cytochrome b_6f complex [14]. The photogeneration of the toxic oxygen species is intensified when the plants are exposed to the stress factors which trouble the use of absorbed light energy for the fixation of the photosynthetic CO_2 [2].

The amino acid sequence from the N-terminal region of the SOD enzyme from various algal species emphasized a homology of 10-15% of the 20 amino acids (Fig. 2).

The phylogenetic inferences of the SOD enzyme emphasize the evolutive relationships between organisms.

Volvox carteri	**M**	**A**	**L**	**S**	**M**	**K**	**V**	**Q**	**A**	**S**	**S**	**L**	**I**	**A**	**G**	**Q**	**R**	**R**	**G**	**R**
Chlorella pyrenoidosa	**M**	**P**	**F**	**Q**	**L**	**P**	**P**	**L**	**P**	**Y**	**A**	**I**	**N**	**A**	**L**	**E**	**P**	**H**	**M**	**S**
Dunaliella salina	**M**	**A**	**A**	**L**	**L**	**S**	**S**	**K**	**L**	**N**	**M**	**T**	**A**	**R**	**P**	**A**	**P**	**A**	**Q**	**R**
Consens	**M**	**A**	-	-	**L**	-	-	-	-	-	-	-	-	**A**	-	-	**P**	-	-	**R**

Fig. 2: The amino acids sequence from the N-terminal sequence of superoxide dismutase in certain green algae

The hydrogen peroxide that derives from superoxide through disproportionation catalyzed by SOD is reduced to water by ascorbate peroxidase using ascorbate as electron donor [20]. The oxidized ascorbate is reduced to ascorbate through ferredoxin, monodehydroascorbate reductase and dehydroascorbate reductase. The activity of ascorbate peroxidase (APX) is located in the cytosol and in the chloroplasts [1]. Four APX isoforms were located in the cytosol and two in chloroplasts. The chloroplasts contain a soluble form of APX in the stroma and a form bound to thylakoids [26]. Five APX isoforms were found in the chloroplasts bound to thylakoids and in the stroma. In the green algae APX acts as an enzyme that removes the hydrogen peroxide: in *Euglena* it is located in the cytosol, while in *Chlamydomonas* it is only in the chloroplast stroma. The cytosolic APX is a hemic enzyme (core Fe^{4+}-O) which removes the hydrogen peroxide through ascorbate oxidation (hemic peroxidases) [23]. The cytosolic and chloroplastic isozymes are different due to instability of the chloroplastic form in the absence of ascorbate.

The ascorbate peroxidase activity was superior to the control in the transition state. In *state 1*, in the presence of DCMU, APX intensifies its activity because in these conditions the activity of PS I is sustained, this photosystem being stimulated by light 1. In *state 2* APX operates at intensities which are higher than in the control, in function of the effects of the applied inhibitor (Fig. 3).

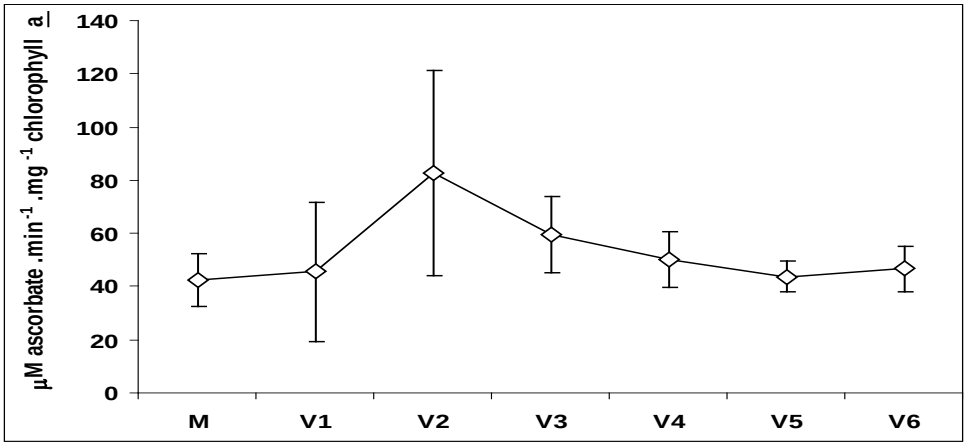

Fig. 3: The activity of ascorbate peroxidase (APX): M = control; *State 1*>V_1=light 1; V_2 – light 1 + DCMU; *State 2* > V_3 – light 2 + FCCP; V_4 – light 2 + FCCP + DCMU; V_5 – light 2 + FCCP + DBMIB; V_6 – light 2 + FCCP + chloramphenicol

The amino acid sequence from the N-terminal region of the APX enzyme from the chloroplasts of various algal species emphasized a homology of 10-15% of the 20 amino acids (Fig. 4).

Chlamydomonas reinhardtii	-	-	-	M	Q	S	A	R	V	S	R	T	A	R	H	T	R	P	S	C
Guillardia theta	-	-	-	F	T	R	P	M	A	V	K	R	A	G	V	R	A	G	R	S
Euglena gracilis	R	S	Y	M	E	K	Y	A	K	D	E	V	A	Y	F	R	D	F	A	T
Chlorella vulgaris	L	Q	P	V	L	I	R	L	G	W	H	D	A	G	T	Y	S	V	E	A
Chlamydomonas sp.	M	-	-	I	G	S	K	I	N	A	P	R	A	S	M	A	S	R	A	A
Consens	-	-	-	M	-	S	-	-	-	-	-	R	A	G	-	R	S	-	A	A

Fig. 4: The amino acids sequence from the N-terminal sequence of ascorbate peroxidase in certain green algae

The catalytic removal of the hydrogen peroxide through APX produces monodehydroascorbate (MDA) which is then nonenzymatically decomposed through spontaneous disproportionation to ascorbate and dehydroascorbate, or through the univalent oxidation of the ascorbate in the enzymatic reactions. In the chloroplast stroma MDA is enzymatically reduced to ascorbate through monodehydroascorbate reductase (MDAR) which uses NADH and NADPH as electron donor. MDA can be also reduced by PS I through ferredoxin pathway [19]. The participation of the cytochrome b_6f and its reductase to the reduction activity of MDA was also pointed out by Hara and Minakami [11]. Monodehydroascorbate reductase is a monomer that contains 1 mole of FAD/mole enzyme which has been reduced through NADPH and reoxidized through monodehydroascorbate. The enzyme has a thiol group whose blockage with reactive thiol inhibits the electron transfer from NADPH to FAD. NADH and NADPH serve as electron donors. In addition to monodehydroascorbate, the enzyme catalyzes the ferricyanure reduction. It is different from NADH dehydrogenase and other enzymes through its molecular weight, its amino acids composition and its specificity for the electron acceptors and donors. MDA reductase is located in the chloroplast stroma [13].

The monodehydroascorbate reductase activity has been enhanced in the state transitions in *Mougeotia* suspension comparatively to the values of control (Fig. 5). In *state 1* and in the presence of DCMU the photochemical activity is sustained only by PS I where the reactive oxygen species are generated, establishing the intensification of the antioxidative enzyme activity. In *state 2* in the presence of specific inhibitors the MDAR activity was amplified comparatively to the control.

The importance of Mehler reaction as regards the removal of peroxide excess was verified by increasing the APX activity as a response to the environmental stress factors. The cell regeneration of ascorbate is accomplished through the direct reduction of MDA radical to ascorbate by MDA reductase using NADPH as electron donor. Correspondinly to this, the MDA radical will spontaneously disproportioned to ascorbate and DHA, in this case, DHA being reduced through DHA reductase and GSH reductase using GSH and NADPH as electron donors. Thus, the presence of this ascorbate/glutathione regenerative system was studied on chloroplasts, but such enzymes also exist in the cytosol. Many enzymes implied in the Calvin cycle are fast inactivated if the hydrogen peroxide concentration is not maintained at low levels by the catalytic activity of APX.

Glutathione reductase (GR) is a flavoprotein which catalyses the reduction of the oxidized glutathione (GSSG to GSH) in the presence of the electron donor NADPH. GR, together with APX and DHAR represent the chloroplastic system for H_2O_2 removal. The GR

activation in located into chloroplasts and it is involved in the glutathione regeneration [7,15]. GR can maintain a high ratio between GSH/GSSG playing significant role in the regulation of cell metabolism. GR in stroma maintains the stoichiometry between the CO_2 release and NADP reduction by G-6-P dehydrogenase [28].

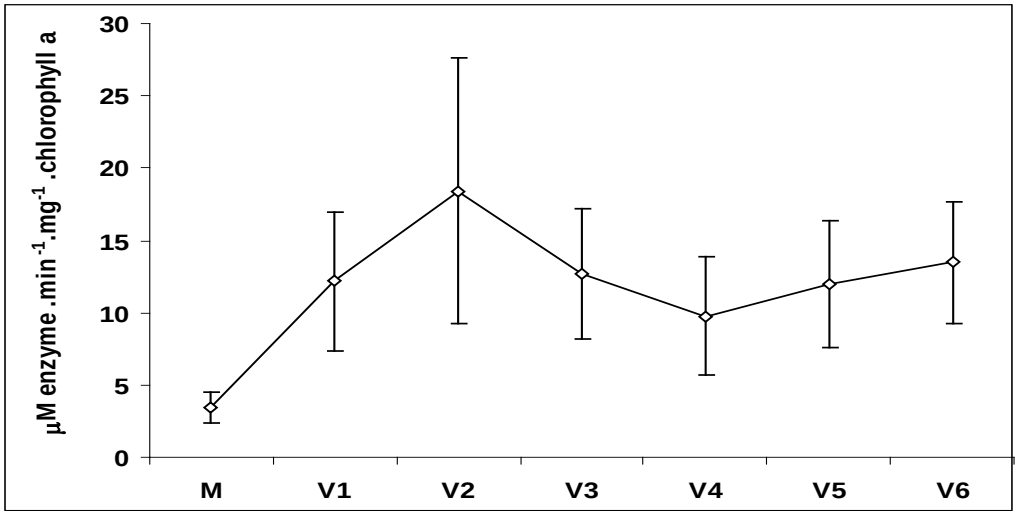

Fig. 5: The activity of monodehydroascorbate reductase (MDAR): M=control *State 1*>V_1=light 1; V_2 – light 1 + DCMU; *State 2* > V_3 – light 2 + FCCP; V_4 – light 2 + FCCP + DCMU; V_5 – light 2 + FCCP + DBMIB; V_6 – light 2 + FCCP + chloramphenicol

The state transitions stimulate the activity of glutathione reductase in the *Mougeotia* suspensions (Fig. 6). In comparison to the control values there was observed the intensification of GR activity especially in *state 1* in the presence of DCMU and in *state 2* in the presence of chloramphenicol.

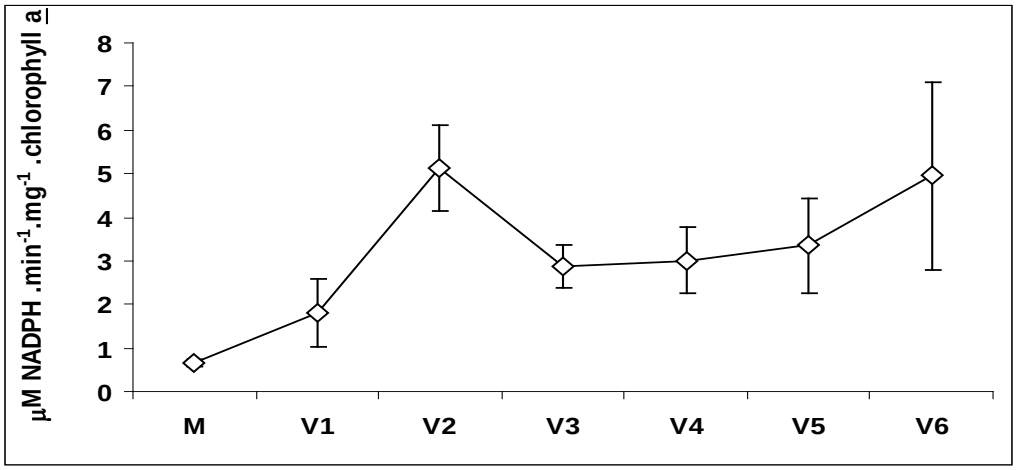

Fig. 6: The activity of glutathione reductase (GR): M = control; *State 1*>V_1=light 1; V_2 – light 1 + DCMU; *State 2* > V_3 – light 2 + FCCP; V_4 – light 2 + FCCP + DCMU; V_5 – light 2 + FCCP + DBMIB; V_6 – light 2 + FCCP + chloramphenicol

NADPH-dehydrogenase is an oxidoreductase which acts on NADPH, the substrate which helps in the CO_2 fixation. The stress factors generate oxygen reactive species by intensifying the NADPH-oxidase activity [24], and this was observed in many plant species [21]. The CO_2 assimilation by oxygenic photosynthesis depends on the NADPH and ATP production through the electron transport leaded by light starting from water to $NADP^+$. In anaerobic conditions ATP was synthesized without changing the NADP/NADPH ratio due to the electron cyclic flow. In

aerobiosis the amount of ATP has enhanced, while the quantity of NADPH has decreased. Once the inhibitors were added, the O_2 evolution was stimulated. These observations suggest that the photosynthetic production of reduced equivalents limits the photosynthetic assimilation [6].

The present results regarding the NADPH dehydrogenase activity have proved the intensification of the activity in state transitions (Fig. 7). Thus, in *state 1* there were obtained significant stimulation in the presence of DCMU and in the *state 2* in the presence of light 2 when the enzymatic activity has enhanced six times, and also in the model with chloramphenicol, were there was observed a five times enhancement comparatively to the control values.

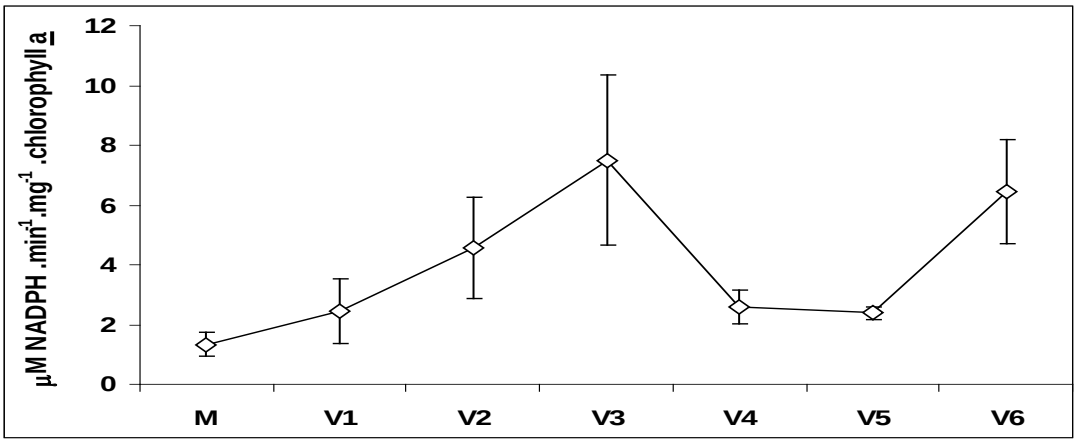

Fig. 7: The activity of NADPH-dehydrogenase: M = control; *State 1*>V_1=light 1; V_2 – light 1 + DCMU; *State 2* > V_3 – light 2 + FCCP; V_4 – light 2 + FCCP + DCMU; V_5 – light 2 + FCCP + DBMIB; V_6 – light 2 + FCCP + chloramphenicol

The Mehler reaction and electron cyclic flow around PS I may contribute to the enhancement of the ATP/NADPH photosynthetic ratio. Under aerobic conditions the NADPH production rate, indirectly emphasized through NADPH-dehydrogenase activity which uses NADPH as enzymatic substrate, is an important process in the photosynthetic assimilation. In the presence of FCCP, the light *2* displays a stimulation of NADP photoreduction by stimulating the activity of NADPH-dehydrogenase.

The lipid peroxidation through lipoxygenase generates singlet oxygen and superoxide anion radicals. The lipoxygenase is a normal enzyme implied in the tissue responses and it catalyses the O_2 reaction with polyunsaturated fatty acids forming the conjugated lipid hydroperoxides. It is possible that the lipoxygenase represents an important initiator of the oxidative damages. The enhancement of its activity is correlated with the high amount of MDA which is a product of the lipid peroxidation. The increase of the lipid peroxidation leads to the enhancement of lipoxygenase activity [8]. The lipids peroxidation dependent by the MDA production was observed in mitochondria and in microsome [10]. The lipid peroxidation leads to the loss of membranes integrity [30].

The inducement of the lipids peroxidation was analyzed by determining the accumulation of the thiobarbituric acid reactive species (TBARS) subsequent to the reaction of thiobarbituric acid in trichloroacetic acid (Fig. 8). In *state 1* in the presence of light, the lipids peroxidation was significantly reduced comparatively to the control values. If the photosynthetic inhibitor DCMU was added, the peroxidation activity of lipids has been doubled. In *state 2*, the lipid peroxidation was significantly reduced because of the blockage of the photosynthetic electron transport and probably of the O_2 production. In all variants the standard deviation was very low.

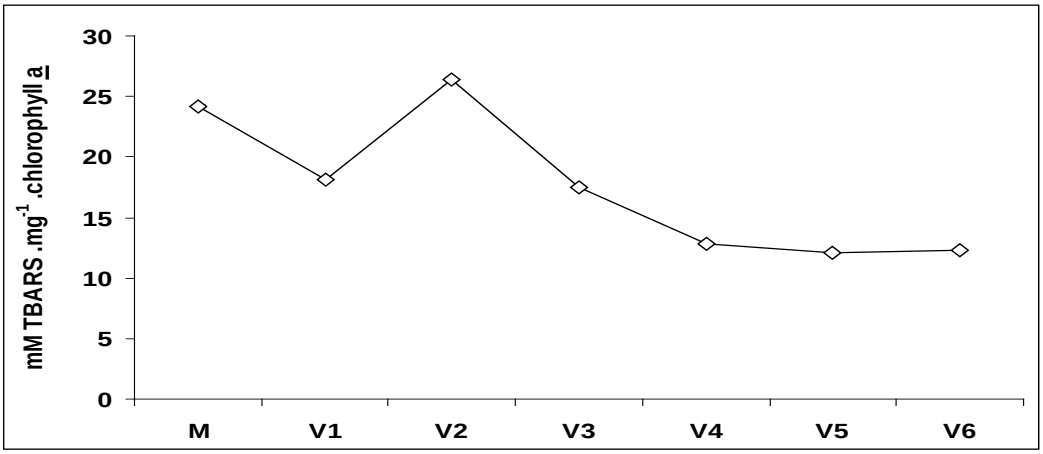

Fig. 8: Lipids peroxidation activity: M=control, *State 1*>V_1=light 1;V_2 – light 1 + DCMU; *State 2* > V_3 – light 2 + FCCP; V_4 – light 2 + FCCP + DCMU;V_5 – light 2 + FCCP + DBMIB; V_6 – light 2 + FCCP + chloramphenicol

The activity of the antioxidative enzymes more or less induced through the photochemical state transitions certifies the fact that the relative flux of the O_2 photosynthetic reducing equivalents through the Mehler reaction is relatively high. The maintenance of the electron flow in the thylakoids in the presence of an adequate $NADP^+$ amount as electron acceptor is essential for the chloroplasts protection against photooxidative stress. In this process involves the cyclic electron flow and the water-water cycle around PS I too [25].

Conclusions

The photoproduction of reactive oxygen species is due to certain physiological and environmental parameters and it is produced when the photonic intensity exceeds the necessary for CO_2 assimilation. These O_2 reactive species are rapidly removed from the inside of the chloroplasts by a group of enzymes consisting in SOD, GR, DHAR, MDHAR and APX.

Superoxide dismutase catalyses the dismutation of the superoxide radicals, and it represents the first step of the enzymatic system for the active oxygen removal. The superoxide dismutase activity has enhanced in both photochemical states, more significantly in *state 1* in the presence of DCMU which leads to the use of electron in PS I toward the deactivation of the oxygen reactive species, by blocking the transport of the photosynthetic electron from PS II. By stopping the PS II, the cyclic electron flow around PS I operates at maximum photosynthesis rate inducing the formation of ATP, in this process being also involved the cytochrome b_6f complex. The hydrogen peroxide which derives from superoxide by SOD catalyzed disproportionation, is reduced to water by ascorbate peroxidase using the ascorbate as electron donor. In *state 1* in the presence of DCMU the ascorbate peroxidase has intensified its activity because in these conditions the activity of PS I is sustained, this being stimulated by light *1*. In *state 2* the ascorbate peroxidase operates at higher intensities and it is related to the effects of the inhibitor used.

The catalytic removal of the hydrogen peroxide through ascorbate peroxidase produces the monodehydroascorbate which is enzymatically reduced to ascorbate by monodehydroascorbate reductase which uses NADH and NADPH as electron donors. The activity of the monodehydroascorbate reductase was modified during the state transitions in the *Mougeotia* suspension, comparatively to the control values.

The importance of the Mehler reaction consists in the maintenance of an ascorbate/glutathione regenerative system in the cell. Glutathione reductase is a flavoprotein which catalyzes the reduction of the oxidized glutathione contributing to the hydrogen peroxide removal. The state transitions have stimulated the glutathione reductase activity especially in

state 1, in the presence of DCMU and in *state 2* in the presence of chloramphenicol. The NADPH-dehydrogenase is an oxidoreductase acting on NADPH, the substrate which helps in CO_2 fixation. The CO_2 assimilation through oxygenic photosynthesis depends on the NADPH and ATP formation through the electron transport leaded by light, starting from water and $NADP^+$. The activity of NADPH dehydrogenase was intensified during the state transitions. Thus, certain stimulation has been obtained during *state 1* in the presence of DCMU and in *state 2,* in the presence of light *2* when the enzymatic activity was higher six times, and in the sample with chloramphenicol where the increase was for five times more intense, comparatively to the control values. The Mehler reaction and the cyclic electron flow around PS I may contribute to the enhancement of the ATP/ NADPH photosynthetic ratio. In aerobic conditions the rate of NADPH generation represents an important step for the photosynthetic assimilation. In the presence of FCCP, light *2* has hastened the $NADP^+$ photoreduction by stimulating the NADPH-dehydrogenase activity. The lipids peroxidation by lipoxygenase generates singlet oxygen and superoxide anion radicals. The inducement of lipid peroxidation was analyzed by determining the accumulation of the thiobarbituric acid reactive species (TBARS) subsequent to the reaction with thiobarbituric acid in trichloroacetic acid. In the state transitions the lipids peroxidation was significantly decreased, while in the presence of DCMU this activity was doubled. The activity of the antioxidative enzymes more or less induced through the photochemical state transitions certifies the fact that the relative flux of the O_2 photosynthetic reducing equivalents through the Mehler reaction is relatively high.

The maintenance of the electron flow in the thylakoids in the presence of an adequate amount of $NADP^+$ as electron acceptor is essential for the protection of chloroplasts against the photooxidative stress In this process participate the cyclic electron flow and the water-water cycle around PS I as well.

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RELAȚIA ÎNTRE CICLUL APĂ-APĂ ŞI TRANZIȚIILE DE STARE LA ALGA VERDE *MOUGEOTIA*, TULPINA AICB 560

(Rezumat)

Activitatea enzimelor antioxidative în timpul tranzițiilor de stare în prezența unor inhibitori specifici la suspensiile de algă *Mougeotia sp.* a fost evaluată. Alga provine din Colecția de culturi de alge a I.C.B. Cluj-Napoca (AICB) şi a fost crescută în soluția nutritivă Bold (BBM), în condiții de agitare continuă cu aer, iluminare continuă cu 630 μmol.m^{-2}.$s^{-1}s^{-1}$, la temperatura de 22^{0}C. Durata de cultivare a fost de 23 de zile.

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: 30 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 (carbonil cianide-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.

Activitatea superoxi-dismutazei a crescut mai semnificativ în *starea 1* în prezența DCMU care determină utilizarea electronilor la nivelul PS I în direcția deactivării speciilor reactive de oxigen. Prin blocarea PS II, curentul ciclic de electroni din jurul PS I operează la rata maximă a fotosintezei. În *starea 1* în prezența DCMU ascorbat peroxidaza şi-a intensificat activitatea datorită activării activității PS I prin lumina *1*. În *starea 2* ascorbat peroxidaza operează la intensități superioare. De asemenea şi activitatea monodehidroascorbat reductazei s-a intensificat în tranzițiile de stare. Tranzițiile de stare au stimulat activitatea glutation reductazei şi NADPH dehidrogenazei în

starea 1 în prezența DCMU şi în *starea 2* în prezența cloramfenicolului. În prezența FCCP, lumina *2* a grăbit fotoreducerea $NADP^+$ prin stimularea activității NADPH-dehidrogenazei.

Peroxidarea lipidelor prin lipoxigenază generează oxigen singlet cât şi radicalii anioni de superoxid. Inducerea peroxidării lipidelor a fost urmărită prin determinarea acumulării speciilor reactive de acid tiobarbituric (TBARS) în urma reacției cu acid tiobarbituric în acid tricloracetic. În tranzițiile de stare peroxidarea lipidelor s-a redus semnificativ, iar în prezența DCMU, activitatea s-a dublat.

Activitatea enzimelor antioxidative indusă prin procesul tranzițiilor de stare atestă faptul că fluxul relativ al echivalenților reducători ai O_2 prin calea reacției Mehler este destul de ridicat, iar prezența $NADP^+$ ca şi acceptor de electroni este esențială, proces la care participă curentul ciclic de electroni şi ciclul apă-apă din jurul PS I.

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