IDENTIFICATION OF PRIMARY PHOTORECEPTORS ADJUSTING THE PHOTOSYNTHETIC APPARATUS BIOENERGETICS TO UV-B RADIATION THROUGH THE CONTROL OF PHOTOSYSTEM II LIGHT HARVESTING COMPLEX

IDENTIFICAREA FOTORECEPTORILOR PRIMARI RESPONSABILI DE AJUSTAREA BIOENERGETICII APARATULUI FOTOSINTETIC LA RADIAȚIA ULTRAVIOLETĂ B PRIN CONTROLUL COMPLEXULUI DE COLECTARE A LUMINII DIN FOTOSISTEMUL II

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Abstract. *Action spectra (from 410 to 730 nm) of the photosynthetic apparatus bioenergetics upon UV-B exposure and during recovery were constructed for the wild type (wt) and a chl-b less mutant (wt-lhc) of Scenedesmus obliquus. Our results indicated three primary photoreceptors, specifically the active and inactive protocholophyllide (620-640/442 nm), a carotenoid absorbing at 535 nm and the reaction center of photosystem I (690-730 nm) as responsible for the tolerance of the photosynthetic apparatus to stress by decreasing the excitation pressure exerted by UV-B on PSII. In contrast, the chlorophylls appeared as primary photoreceptors responsible for the enhanced sensitivity of the photosynthetic apparatus to UV-B. These data suggest that the photosynthetic apparatus sensitivity or tolerance to UV-B radiation could be modulated through the excitation of certain photoreceptors which alters the antenna size and, subsequently, the amount of energy used in photosynthesis or dissipated as heat or fluorescence. In addition, our results indicate the LHCII as a key element for the restoration of the photosynthetic apparatus functionality after UV-B stress.*

Key words: photosynthetic apparatus; UV-B stress*; Scenedesmus obliquus*

Rezumat. *Spectre de actiune (de la 410 la 730 nm) ale bioenergeticii aparatului fotosintetic au fost construite pentru tipul salbatic si o mutanta deficitara in clorofila b de Scenedesmus obliquus, atat pe parcursul cat si dupa expunerea la radiatia ultravioleta B (UV-B). Rezultatele obtinute indica trei fotoreceptori primari si anume: protoclorofilidul activ si inactiv cu absorbtie la 620-640/442 nm, un carotenoid ce absoarbe la 535 nm si centrul de reactie al fotosistemului I cu absorbtie la 690-730 nm ca fiind responsabili pentru toleranta aparatului fotosintetic la UV-B prin scaderea presiunii de excitatie exercitata asupra fotosistemului II. In contrast, clorofilele sunt responsabile pentru senzitivitatea crescuta a aparatului fotosintetic la UV-B. Aceste date sugereaza ca senzitivitatea sau toleranta aparatului fotosintetic la UV-B poate fi modulata prin excitarea anumitor fotoreceptori care altereaza marimea complexului de colectare a luminii (antena) din fotosistemul II si, in consecinta, cantitatea de energie ce poate fi folosita in fotosinteza sau disipata sub forma de caldura sau fluorescenta. In plus, rezultatele noastre indica antena ca fiind un element cheie pentru restaurarea functionalitatii aparatului fotosintetic dupa expunerea la stresul cauzat de UV-B.*

Cuvinte cheie: aparat fotosintetib; UV-B stres*; Scenedesmus obliquus*

INTRODUCTION

Ozone depletion due to the pollution of stratosphere by CFC (chlorofluorocarbons) had stimulated the interest for the study of the increased UV-B radiation (280-320 nm) impact on the photosynthetic apparatus. The photosynthesis sensitivity to UV-B or other abiotic stresses depends on the regulation of the balance between the energy absorbed and that used in photochemistry or dissipated as heat. In these processes the light-harvesting complex of PSII (LHCII) has a primordial role. Its role is not only restricted to the capture of photons but it also protects the photosynthetic apparatus against excessive energy flow by dissipating it through a mechanism called non-photochemical quenching (NPQ). Recently, we demonstrated that UV-B tolerance can be induced by decreasing the LHCII size (Sfichi et al., 2004). However, this does not mean that a photosynthetic apparatus lacking the LHCII is more tolerant to UV-B than one characterized by a big antenna. During the investigations of UV-B responses in two strains of green algae *Scenedesmus obliquus*, the wild type (wt) and a mutant lacking the LHCII (*wt-lhc*) we found that the LHCII is absolutely required for the recovery from UV-B effects (Sfichi-Duke et al., 2008). Interesting is also the influence of visible light intensity used as background during UV-B irradiation experiments on photosynthetic responses. For instance, in low-light conditions, the LHC size and energy dissipation rate are high and the photosynthetic efficiency is low. Inverse responses are obtained in high-light conditions (Sfichi et al., 2004). Since the response to UV-B radiation is affected by the photoadaptation status to visible light, the investigation of the primary photoreceptors involved in these responses could provide additional information regarding the contribution of antenna to UV-B sensitivity. For this reason, action spectra (from 410 to 730 nm) of the photosynthetic apparatus bioenergetics in UV-B stress conditions and after additional recovery were constructed for two strains of *Scenedesmus obliquus*, the wild type (wt) and a chlorophyll *b*-less mutant (*wt-lhc*).

MATERIALS AND METHODS

Organism and growth conditions

Cultures of the unicellular green alga *Scenedesmus obliquus*, the wild type and a *wt-lhc* mutant were autotrophically grown in liquid culture medium (Bishop and Senger, 1971) into a temperature-controlled water bath (30^0C).

UV-B treatment and recovery

The wild type and *wt-lhc* cultures were exposed for 3 h in dark (D), white light (WL) or in 20 different wavelengths of monochromatic light (ML) of equal intensity (15 μmol m^{-2} s^{-1}). After 3 h of photoadaptation, the UV-B treatment was performed for 90 min using a dose of 0.420 mW cm^{-2} at the surface of the culture. After the cessation of UV-B treatment, the cultures were let for recovery in the same light conditions as those used during UV-B treatment. The UV-B effect was assessed by expressing the values obtained after 90 min of UV-B irradiation as % of the corresponding control values (measured prior to UV-B irradiation). To quantify the LHCII contribution the values obtained for the *wt-lhc* mutant cultures were extracted from those obtained for the wild type. The calculated difference ("Δ") indicated the primary photoreceptors, responsible for the attenuation or amplification of the UV-B effect on the

photosynthetic apparatus by sensing the LHCII. Similarly, the recovery ability regulation after UV-B exposure was also investigated.

Chlorophyll *a* fluorescence measurements

Rapid Chl *a* fluorescence transients were recorded at room temperature with high time-resolution PEA (Plant Efficiency Analyzer) fluorometer (Hansatech Ltd., King's Lynn, Norfolk, UK) as previously described by Sfichi et al. (2004). Chl fluorescence kinetics was analyzed with PAM (Pulse Amplitude Modulated) fluorometer (P200, Heinz Walz). The fluorescence Fo was measured by using a modulated light (ML) with a low intensity (1 μmol m^{-2} s^{-1}) The maximal fluorescence yield, Fm, was induced by a short saturating pulse (SP) of white light (3000 μmol m^{-2} s^{-1}). The maximal fluorescence yield Fmwas induced by SP given periodically a t every 30 s during continuous exposure to AL. At the steady state of electron transport, the AL was turned off and a far-red light (FR) was applied to ensure rapid oxidation of Q_A and the fluorescence Fo' was measured. Parameters such as qP, qN and Φs_{PSII} have been calculated according to Maxwell and Johnson (2000).Q_B non-reducing centers were calculated according to Melis and Homann (1976).

Determination of the packed cell volume (PCV)

The PCV of a cell suspension was determined as described in Sfichi et al. (2004).

RESULTS AND DISCUSSIONS

Figure 1 shows the polyphasic kinetics of the Chl fluorescence rise from F_0 (O level) to F_m (P level) of a Kautsky curve (Strasser and Strasser, 1995) under white light. Both wild type and *wt-lhc* mutant UV-B irradiated cultures exhibited a significant decrease of the J-P phase. In the wild type, the fluorescence yield recovered within 4 h after the cessation of UV-B treatment. In contrast, there was an irreversible lost of fluorescence yield in the *wt-lhc* mutant.

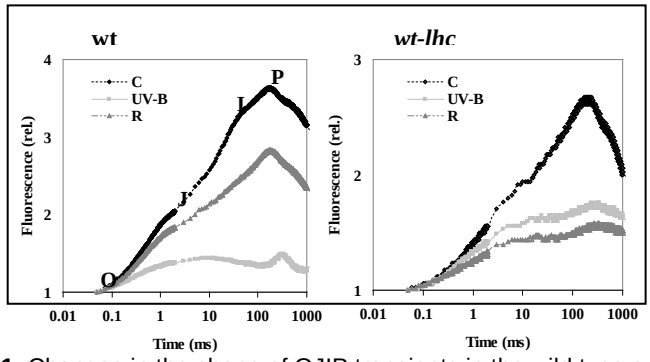

Fig.1. Changes in the shape of OJIP transients in the wild type and wt-lhc mutant prior to UV-B irradiation (C), after 3h of UV-B irradiation (UV-B) and after 4 h of additional recovery (R)

The action spectrum obtained for the maximum quantum yield of PSII (Fv/Fm) showed 3 negative peaks (430, 550 and 656 nm) suggesting that mainly chlorophylls (Chl, 656/430 nm) and some carotenoids (Car, 550 nm) from the LHCII could be responsible for this down-regulation. The negative effect of UV-B on the Fv/Fm was reversed at 442, 535 and 620-640 nm. Probably, the two forms of protochlorophyllide (PChlide): free PChlide (620/442 nm) and active PChlide

(640/442 nm) (POR-PChlide-NADPH complex) were involved in the reduction of UV-B effect on the Fv/Fm. There was no significant effect on the Fv/Fm in the (far)red region of the spectrum, suggesting that neither the reaction centers of PSII nor the PSI were involved in the regulation of UV-B effect on the Fv/Fm (Fig. 2A).

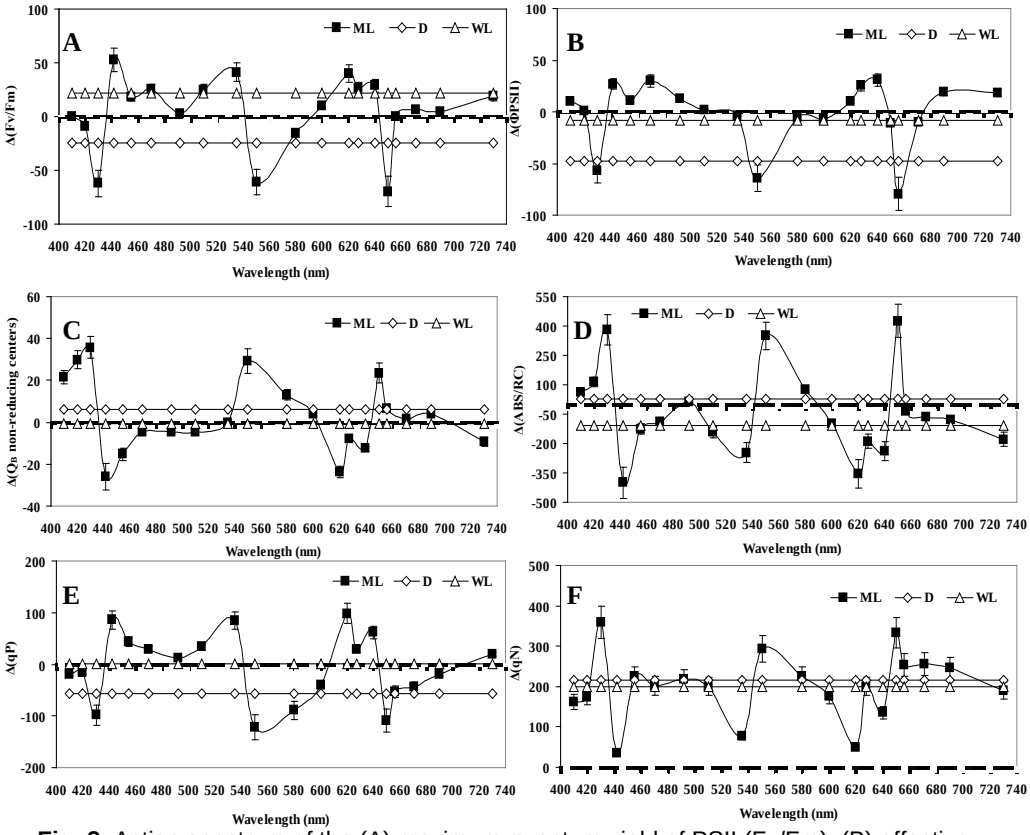

Fig. 2. Action spectrum of the (A) maximum quantum yield of PSII (Fv/Fm), (B) effective quantum yield of PSII (ΦPSII), (C) QB non-reducing centers, (D) functional antenna size (ABS/RC), (E) photochemical quenching (qP) and (F) non-photochemical quenching (qN) after exposure to UV-B radiation.

UV-B radiation also induced the decrease of the operational quantum yield efficiency of PSII (Φ_{PSII}). Upon excitation of PChlides, there was a reduction of the UV-B effect. In contrast, a strong decline in the Φ_{PSII} was found upon irradiation with 656 and 430 nm, suggesting that Chls enhanced the UV-B effect. Reduction of UV-B induced down-regulation of Φ_{PSII} was also seen upon excitation with far red (730 nm) light, suggesting the involvement of PSI in the stimulation of LHCII efficiency (Fig. 2B). In parallel to the down-regulation of quantum yield efficiencies (Fv/Fm and Φ_{PSII}) by UV-B there was an increase in the amount of Q_B non-reducing centers. The action spectrum showed clearly that Chls (656/430 nm) were the primary photoreceptors mediating this effect. In contrast, the stimulation of PChlides (620-

640/442 nm) and PSI (730 nm) preserved the functionality of reaction centers (Fig. 2C).

Light absorbed by Chls (656/430 nm) also increased the functional antenna. As result, there was a decrease in the photochemical quenching (qP) of light energy. Inverse effects were obtained for wavelengths absorbed by PChlides (620-640/442 nm) (Fig. 2D-E) when the qN capacity was decreased (Fig. 2F). Recovery occurred only in the wild type cultures; exception was found for the cultures incubated in light of 656 and 550 nm, which did not recover (data not shown).

The action spectra confirmed our previous finding about the correlation existent between the antenna size and the sensitivity to UV-B (Sfichi et al., 2004). At wavelengths where antenna is small, the photosynthetic efficiency is increased and *vice-versa*. By altering the antenna size, the balance between light energy absorbed versus energy utilized in photochemical reactions (qP) or dissipated as heat (qN) could be regulated. For instance, upon excitation with ML of 442 nm and 620-640 nm there is an increase in the qP in parallel to the diminution of qN. The photoreceptors sensitized by these wavelengths are the free protochlorophyllide (inactive PChlide absorbing in 620/442 nm) and the "active" Pchlide (absorbing in 640/442 nm). Probably, they are involved in the capture of the excitation energy but they do not transfer this energy to chlorophylls of the LHCII (because they are not constituents of the LHCII) and therefore, they contribute to the minimization of the PSII over excitation. Also, it is possible that at least a part of the excitation light energy absorbed by the active PChlide is used for its conversion to chlorophyllide (Chlide) (Kotzabasis et al., 1990). In this way, the PSII excitation pressure (described as 1-qP) is decreased and the quantum yield efficiencies of PSII increased. Additionally to PChlides, the action spectra revealed the possible involvement of a carotenoid, which by excitation at 535 nm leads also to an increase in the photochemical quenching capacity, probably due to the efficiency of light capture. A high qP also expresses a high ability to produce energy sources that can be used for repair and the cell can further cope with the enhanced UV-B. As result of excitation pressure decreasing, the antenna size also decreased in order to minimize the energetic losses through the non-photochemical quenching. Consequently, upon the excitation of certain photoreceptors (PChlides, PSI and Car) with adequate ML (620-640/442, 535 and 700 nm) the photosynthetic apparatus became more tolerant to UV-B radiation.

According to our previous works (Sfichi et al., 2004) two mechanisms may contribute to the increase in the antenna size upon UV-B irradiation: i) the increase in the LHCII size and ii) the transformation of the Q_B non-reducing centers in dissipative sinks for the excitation energy. While in the wild type culture, both mechanisms are found upon UV-B irradiation, in the *wt-lhc* mutant only the second one is present (Sfichi-Duke et al., 2008). However, this mechanism could not offer enough protection to UV-B since the mutant did not recover after the treatment. As previously demonstrated (Sfichi-Duke et al., 2008), recovery requires the participation of the LHCII which upon UV-B treatment increases its oligomerization state. The LHCII trimers have a high potential for energy dissipation (Pascal et al., 2005). Probably, the changes in the LHCII upon UVB treatment contribute to conformational re-adjustments of

PSII units within the thylakoid membranes that assure not only the dissipation of excess energy but also to the repair of damage. It seems that the importance of LHCII in the mechanism determining the degree of sensitivity to UV-B consists not only of its capacity to keep the balance between energy captured and used at reaction centers, but also it may be involved in structural re-adjustments that stabilize the thylakoid membranes in UV-B stress conditions.

CONCLUSIONS

Based on the preceding data, the main conclusions that can be drawn here are:

1. A higher excitation pressure is compensated by an efficient system of photochemical/non-photochemical energy quenching that is assured in the presence of LHCII.

2. A photosynthetic apparatus without LHCII has no potential to recover the damage induced by UV-B.

3. Three primary photoreceptors (active and inactive PChlide, carotenoids absorbing at 535nm and the reaction center of PSI) increase the tolerance of the photosynthetic apparatus to UV-B by decreasing the excitation pressure exerted on PSII by UV-B.

4. Chlorophylls (Chl *a* and *b*) are primary photoreceptors responsible for the enhanced sensitivity of the photosynthetic apparatus to UV-B radiation.

Acknowledgements: We heartily thank Prof. Norman Bishop (Oregon State University, USA) for kindly providing the *wt-lhc* mutant culture.

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