Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 66, No 6, 2013

BIOLOGIE

Biophysique

SENSITIVITY OF TWO ECOTYPES OF *ARABIDOPSIS THALIANA* (Cvi AND Te) TOWARDS UV-B IRRADIATION

Maya Velitchkova, Daniela Stanoeva, Antoaneta V. Popova

(*Submitted by Academician K. Koumanov on February 12, 2013*)

Abstract

The susceptibility of *Arabidopsis thaliana* towards the detrimental effect of UV-B irradiation was investigated using two ecotypes, Cvi and Te. The effect of UV-B treatment on primary photosynthetic reactions – energy interaction between the main pigment-protein complexes and oxygen evolution, was evaluated at low (4 °C) and at room (22 °C) temperature. UV-B-induced alterations of investigated photosynthetic reactions are better expressed at 22 °C than at 4 °C for Cvi. For Te ecotype the energy interaction was suppressed to higher extent at 22 °C, while oxygen evolving activity was affected similarly at both temperatures. At low and room temperature, the energy interaction in the complex PSII-core antenna is affected stronger by UV-B treatment than the energy distribution between both photosystems, as revealed by fluorescence ratios of 77 K spectra. The results presented indicate that the *Arabidopsis thaliana* ecotype Cvi (Cape Verde Islands) is less affected by UV-B irradiation in respect to the investigated primary photosynthetic reactions than the ecotype Te (Finland).

Key words: ecotypes of *Arabidopsis thaliana*, UV-B radiation, 77 K fluorescence, flash oxygen yields

Introduction. During their development, photosynthetic organisms – green algae, cyanobacteria, higher plants, are subjected to different environmental stress conditions as high and low temperature, high light intensity, salinity, dehydration, UV irradiation that influence negatively their growth and productivity. The sun electromagnetic radiation in the region of 200–400 nm (UV light) represents only seven per cent of the whole spectrum. The ozone layer in the stratosphere absorbs all of the solar UV-C (< 280 nm) and part of UV-B (280–320 nm) radiation, but

This work was performed under a project with the Bulgarian Academy of Sciences 2011–2013.

due to human activities the ozone layer is continuously reducing, thus the amount of UV-B radiation reaching the surface of the Earth is constantly increasing. The levels of solar UV-B radiation on earth vary markedly with the latitude, altitude, season and time of the day [1]. Plants distributed along lower latitudes or higher elevations, where UV-B radiation is higher, possess more pronounced adaptive mechanisms than those from higher latitudes and/or lower elevations [2].

The deleterious effect of UV-B radiation on living organisms is expressed by damaging the biological macromolecules; DNA, proteins and lipids [3], which results in cell death, mutagenesis and cell transformations. In plants UV-B radiation has a pronounced damaging effect on DNA, membranes and photosynthetic processes [1]. Damages affecting photosynthesis concern mainly PSII, the manganese cluster of the water splitting complex, disruption of thylakoid membranes, reduction of chlorophyll content, disturbance of membrane permeability, reduction of the activity and amount of Rubisco and other enzymes [1, 2, 4]. In addition, it has also been reported that PSII, in respect to its location, demonstrates different sensitivity towards UV-B radiation, the grana situated PSII being more sensitive than the stroma situated ones [5]. The most sensitive part of the whole photosynthetic apparatus is the manganese cluster attached to the lumen exposed side of PSII that has been reported to be the first target of UV-B attack [6]. It is supposed that in addition to the primary damaging effect of UV-B radiation a formation of reactive oxygen species (ROS) is induced, that react with lipids, pigments, proteins and nucleic acid [7, 8], thus causing secondary oxidative injury [2, 9]. To cope with the deleterious effect of UV-B radiation, photosynthetic organisms respond by balancing between variety of damaging reactions by both repair and acclimation mechanisms.

Arabidopsis thaliana is a small crucifer, annual weed, that is spread all over Europe and central Asia, with latitudinal range of 68 °N to 0° [10] and is acclimated to a wide range of climatic conditions. It has attracted a lot of scientific interest and is often used as a model high plant for performing classical and molecular genetic studies [11].

The aim of the present study was to evaluate the sensitivity of two ecotypes of *Arabidopsis thaliana*, Cvi and Te, towards UV-B irradiation. The ecotype Cvi (Cape Verde Islands), northern latitude of origin of 16°, is chosen because it grows naturally at substantially higher UV levels, while Te (Finland) grows at latitude of 60° [12], where the levels of UV radiation are lower [3]. To reach this goal, isolated thylakoid membranes from both ecotypes were subjected to UV-B radiation and alterations in the primary photosynthetic reactions, energy transfer between the main pigment-protein complexes and activity of oxygen-evolving centers, were determined. The dependency of UV-B-induced effect on the temperature during irradiation – low (4 °C) or room (22 °C) temperature, was also evaluated.

Materials and methods. Growth conditions of the plant material. The two ecotypes of *Arabidopsis thaliana* (Cvi and Te) were grown on soil

in a growth chamber with 12 h photoperiod (22 °C day/18 °C night temperature) at 100 µmol m^{-2} s^{-1} photon flux density. For isolation of thylakoid membranes, 21–28-day-old plants (Cvi) and 45-day-old plants (Te) with fully developed rosettes were used [11].

Isolation of thylakoid membranes. Thylakoid membranes were isolated from the two ecotypes by the method described in [13]. Briefly, rosette leaves were grinded in 20 mM TRICINE buffer (pH 8.4), 10 mM $NaHCO_3$, 5 mM EGTA, 5 mM EDTA, 0.33 M sucrose. The homogenate was filtered through miracloth and centrifuged for 5 min at 3000 × g. The chloroplasts were washed and resuspended in 20 mM HEPES (pH 7.6) 10 mM $NaHCO_3$, 5 mM $MgCI_2$, 2.5 mM EDTA and 0.3 M sucrose.

UV-B treatment. Isolated thylakoid membranes were irradiated with UV-B (312±25 nm) fluorescent tubes (TL 20 W/12 R, Philips, Hamburg, Germany) at low (4 °C) or room temperature (22 °C) for 60 min. As controls were used non-irradiated thylakoid membranes at the respective temperature. The biological effectiveness of UV-B radiation (UVBBE) was 14.4 kJ m^{-2} d^{-1}. All samples contained thylakoid membranes equivalent to 150 µg chl/ml dissolved in a reaction medium containing: 20 mM MES (pH 6.5), 5 mM $MgCl_2$, 10 mM NaCl and 0.33 M sucrose.

Measurement of oxygen evolution. Determination of oxygen flash yields and initial oxygen burst was performed by home-constructed equipment described in detail by Zeinalov [14] and all details for the experimental setup were described in details in [15]. The values for Y_3 (amplitude of the third flash) and A (amplitude of the initial oxygen burst at continuous illumination) were measured directly from the experimental traces [16]. Thylakoid membranes were dissolved in a reaction medium containing: 20 mM MES (pH 6.5), 5 mM $MgCl_2$, 10 mM NaCl and 0.33 M sucrose. In no case artificial electron acceptor was added.

Low temperature fluorescence measurements. Emission fluorescence spectra at low temperature (77 K) were recorded as described in [17]. Thylakoid membranes were dissolved in buffer containing 20 mM HEPES (pH 7.6), 5 mM $MgCl_2$, 10 mM $NaHCO_3$, 2.5 mM EDTA and 0.33 M sucrose to a concentration of 15 µg chl/ml. Spectra were analyzed by Origin 7.0 after subtraction of the baseline.

Results and discussion. In order to unravel the sensitivity of two ecotypes of *Arabidopsis thaliana* (Cvi and Te) towards UV-B radiation, the alterations in the photosynthetic apparatus in respect to energy interaction of the main pigment-protein complexes and oxygen evolving activity, isolated thylakoid membranes from both ecotypes originating from areas receiving different UV-B dose were investigated. Thylakoids were irradiated with UV-B at 4 °C and 22 °C for 60 min and results were compared with such obtained for control, non-irradiated thylakoids.

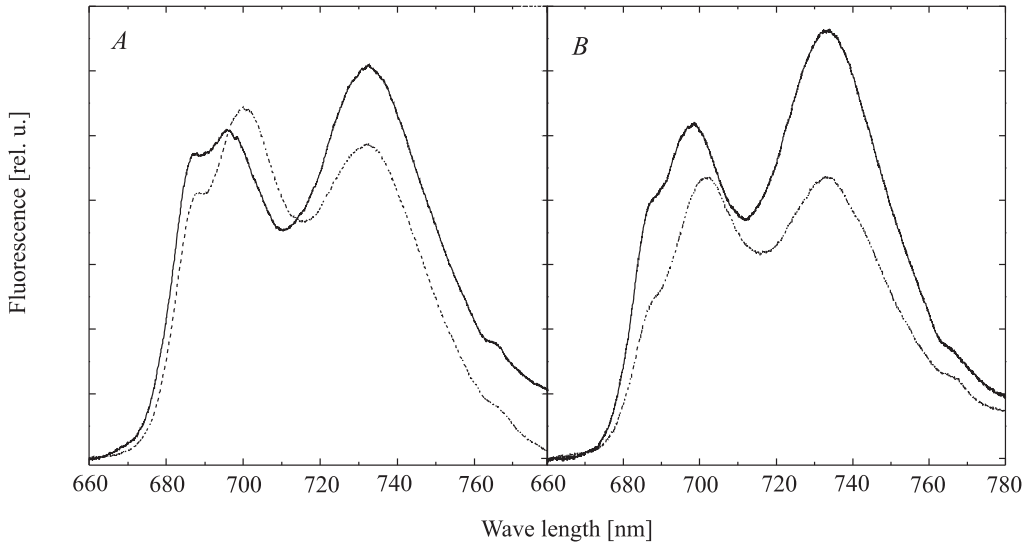

Fig. 1. 77 K Fluorescence emission spectra of *Arabidopsis thaliana* thylakoid membranes, isolated from Cvi (*A*) and Te (*B*). Freshly isolated thylakoid membranes – solid line and irradiated with UV-B at 4 °C for 60 min – dashed line. Fluorescence was excited at 436 nm, chlorophyll concentration – 15 µg chl/ml, slits – 4 nm. Spectra are presented after subtraction of baseline

With the aim of investigating the UV-B-induced alterations in the energy distribution and energy interaction between the main pigment-protein complexes, fluorescence emission spectra at low temperature (77 K) of control and UV-B irradiated thylakoid membranes at both temperatures were recorded. In Figure 1 the fluorescence emission spectra of non-irradiated thylakoid membranes and of UV-B irradiated for 60 min at 4 °C are presented, from Cvi (Fig. 1*A*) and Te (Fig. 1*B*). The alterations in thylakoid membranes, irradiated with UV-B at 22 °C are similar to those at 4 °C, but expressed stronger, and for simplicity only the spectra at low temperature are given. As a result of UV-B irradiation at 4 °C, the overall emitted fluorescence, as judged by the area under the fluorescence contour, is decreased with about 4% and 12%, for Cvi and Te ecotypes, respectively. In the fluorescence spectra of thylakoid membranes at 77 K, three main peaks are displayed, at 685, 695 and 735 nm. The first two peaks, F685 and F695 have been shown to be emitted by the reaction centre of photosystem II (PSII) and its core antenna [18] respectively, while the peak F735 is attributed to the fluorescence emitted by photosystem I (PSI) and its proximal antenna [19]. Alterations in the ratios of relative heights of the three fluorescence peaks (F685/F695 and F735/F685) indicate changes in the energy interaction in the multi pigment-protein complex of PSII and energy distribution between PSII and PSI, respectively. The two fluorescence ratios, F685/F695 and F735/F685, of thylakoid membranes kept for 60 min (non-UV-B treated) and UV-B irradiated

Table 1

Fluorescence ratios, F685/F695 and F735/F685, of thylakoid membranes isolated from *Arabidopsis thaliana*, Cvi and Te. Thylakoids were irradiated with UV-B for 60 min at 4 °C or at 22 °C (+UV − B) or non-irradiated (−UV − B) for the same period of time. Ratios were calculated from 77 K fluorescence spectra after subtraction of the baseline. Fluorescence was excited at 436 nm, spectra recorded in the region 660 – 780 nm, slits – 4 nm. Values are mean from three independent experiments and SE was less than 5%

		4 °C		22 °C	
		F685/F695	F735/F685	F685/F695	F735/F685
Cvi	(−UV − B)	0.926 (100%)	1.193 (100%)	0.882 (100%)	1.166 (100%)
	(+UV − B)	0.756 (82.18%)	1.182 (99.05%)	0.573 (64.9%)	1.513 (129.8%)
Te	(−UV − B)	0.799 (100%)	1.678 (100%)	0.790 (100%)	1.550 (100%)
	(+UV − B)	0.568 (71.14%)	1.762 (105.3%)	0.366 (46.4%)	2.183 (140.8%)

at 4 °C or at 22 °C are arranged on Table 1. The alterations in both ratios, for Cvi and Te ecotypes, are more pronounced for thylakoids illuminated with UV-B at 22 °C when compared with that at 4 °C. Similar UV-B-induced higher effect at 22 °C was obtained for *Arabidopsis thaliana* (C24) (unpublished results). The ratio F685/F695, informative of the energy interaction between the reaction centre of PSII and CP43 and/or CP47, is decreased for samples illuminated with UV-B due to the relative increase of the peak F695 due to disconnection of CP43 and/or CP47 from the reaction centre of PSII. It is worth noting that even for control, non-UV-B treated membranes from *Arabidopsis thaliana*, the emission at 695 nm is higher than that at 685 nm, which could be a species peculiarity, as for thylakoid membrane from spinach, pea and other higher plants under optimal conditions the emission at 685 nm is usually higher. This specificity is most probably related to different interaction between the reaction centre of PSII and CP47 and/or CP43 [18]. The UV-B-induced decline of F685/F695 for UV-B irradiation at 4 °C is with 18% and with 30% for Cvi and Te thylakoids, respectively. The fluorescence ratio F735/F685 is insignificantly altered for thylakoid membranes irradiated with UV-B at 4 °C. These results indicate that the UV-B-induced alterations concern mainly the energy distribution within the PSII-core antenna complex for both investigated temperatures and is valid for both investigated ecotypes, while the energy delivery to PSI is insignificantly affected at low temperature but stronger for irradiation at room temperature. The sensitivity of Te thylakoid membranes towards irradiation with UV-B is higher than for Cvi thylakoids.

Having in mind that the UV-B treatment affects stronger the energy interaction in the multi pigment-protein complex of PSII, further we investigated the UV-B-induced alterations in this complex in respect to its ability to evolve oxygen. The oxygen evolving complex, attached to the lumen exposed side of PSII,

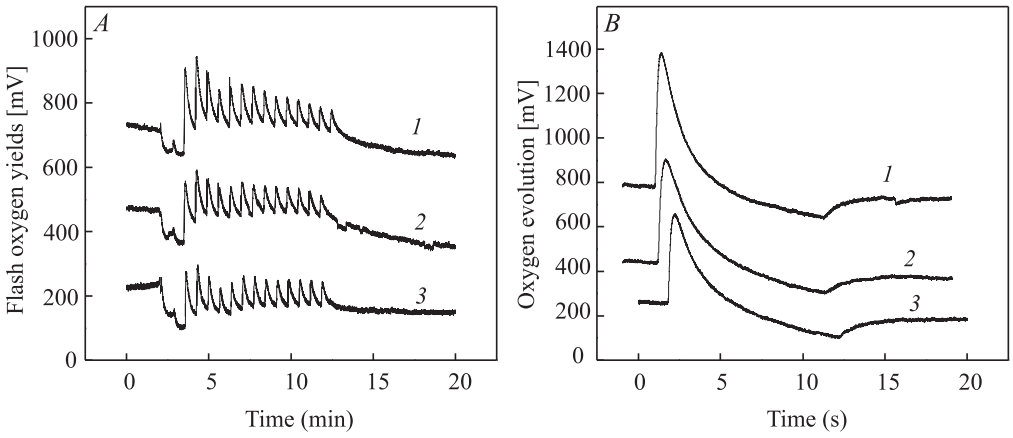

Fig. 2. Typical oscillation pattern of oxygen flash yields of thylakoid membranes, isolated from *Arabidopsis thaliana*, ecotype Cvi, measured without exogenous electron acceptor (*A*). *1* – traces of oxygen flash yields of thylakoid membranes, 60 min, without UV-B treatment at 4 °C, *2* – after 60 min irradiation with UV-B at 4 °C, *3* – after 60 min irradiation with UV-B at 22 °C. Typical traces of initial oxygen burst at continuous irradiation of dark adapted thylakoid membranes, isolated from *Arabidopsis thaliana*, ecotype Cvi, measured without exogenous electron acceptor (*B*). Curves *1*, *2* and *3* – as in (*A*)

is believed to be the most sensitive part of photosynthetic apparatus towards environmental stress conditions [6]. In Figure 2 are presented the typical traces of the oscillation pattern of oxygen flash yields (Fig. 2*A*) and of oxygen burst kinetics at continuous illumination (Fig. 2*B*) of thylakoid membranes isolated from Cvi obtained from non-treated and from UV-B irradiated for 60 min at 4 °C and 22 °C. The results obtained after UV-B irradiation of thylakoid membranes from Te follow the same tendency (data not shown).

After illumination with periodic short flashes, a typical oscillation pattern of oxygen flash yields is obtained, where the amplitude of the yield from third flash (Y_3) is the highest. Analyzing the so obtained oscillation pattern of oxygen evolution, information about the functioning of PSII reaction centres, situated in the grana thylakoid regions (PSIIα) and/or about injuries suffered by these complexes can be obtained (Fig. 2*A*).

The initial oxygen burst at continuous illumination is followed by a biphasic exponential decay (Fig. 2*B*). It is believed that the oxygen evolution is due to functioning of two mechanisms – non-cooperative and cooperative. It is supposed that the grana situated PSII (PSIIα) centres evolve oxygen by non-cooperative mechanism; each reaction centre operates independently and evolves one molecule oxygen after four successive photoreactions. Contrary, the reaction centres, situated in the stroma thylakoids (PSIIβ) evolve oxygen by cooperation of oxygen precursors obtained in different reaction centres, the so-called cooperative mechanism [20]. The values for Y_3 and amplitude of the initial oxygen burst A of

Table 2

Parameters of oxygen evolution of thylakoid membranes of *Arabidopsis thaliana*, ecotypes Cvi and Te, after UV-B irradiation for 60 min at 4 °C or at 22 °C (+UV − B), expressed as per cent from non-irradiated controls (−UV − B). Y_3 – amplitude of the third oxygen flash yield, calculated from the oscillation pattern of thylakoid membranes, illuminated with short saturating flashes, calculated from the results presented in Fig. 2*A* – amplitude of initial oxygen burst at continuous illumination, calculated from results presented in Fig. 2*B*. Values are mean from three independent experiments and SE is less than 5%

		4 °C		22 °C	
		Y_3	A	Y_3	A
Cvi	(−UV − B)	100%	100%	100%	100%
	(+UV − B)	72%	80.4%	59.3%	57.7%
Te	(−UV − B)	100%	100%	100%	100%
	(+UV − B)	50%	19.15%	50%	19.95%

thylakoid membranes irradiated with UV-B for 60 min, expressed as percentage from the respective control (non-irradiated), are summarized on Table 2.

UV-B irradiation leads to a decline in the values for Y_3 and A at both temperatures during irradiation, indicating damage of the oxygen evolving capacity of PSII centres. The amplitude of the third flash (Y_3) for Cvi thylakoid membranes is decreased with 28% and 40%, when irradiation is performed at low or at room temperature, respectively. The amplitude of the initial oxygen burst at continuous illumination for Cvi thylakoids is decreased to a comparable extent, with 20% and 42%, at low or room temperature, respectively. The alterations in these two parameters, Y_3 and A, for Te membranes, are practically identical for both investigated temperatures during UV-B irradiation – decline with 50% for Y_3 and with 80% for A.

Conclusions. The results presented here indicate that UV-B-induced alterations in energy interactions between the main pigment-protein complexes and oxygen evolving functions of thylakoid membranes of *Arabidopsis thaliana*, ecotypes Cvi and Te, are stronger expressed when irradiation is performed at room temperature than at 4 °C. The higher UV-B effect at 22 °C when compared with that at 4 °C can be due either to UV-B-induced generation of different ROS, or to its acceleration at the higher temperature. The energy interaction in the complex PSII-core antenna is influenced stronger than the energy delivery towards PSI. The oxygen evolving capacity of PSII of Te is more sensitive to the detrimental UV-B radiation than Cvi. In summary, the ecotype Te originating from 60° latitude, is more sensitive towards UV-B irradiation than Cvi ecotype, native to 16° latitude. An explanation can be that Cvi is more resistant to UV-B radiation as it is acclimated to higher doses of such radiation than Te.

Acknowledgements. The seeds of the two ecotypes of *Arabidopsis thaliana*, Cvi and Te, were a generous gift from Dr. Hincha DK, Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany.

REFERENCES

[1] Rozema J., J. van de Staaij, L. O. Bjorn, M. Caldwell. Trends in Ecology and Evolution, **12**, 1997, No 1, 22–28.

[2] Caldwell M. M., C. L. Ballare, J. F. Bornman, S. D. Flint, L. O. Bjorn, A. H. Teramura, G. Kulandaivelu, M. Tevini. Photochem. Photobiol. Sci., **2**, 2003, No 1, 29–38.

[3] Jansen M. A. K., V. Gaba, B. M. Greenberg. Trends in Plant Science, **3**, 1998, No 4, 131–135.

[4] Vass I., A. Szilard, C. Socora. In: Handbook of Photosynthesis, 2nd ed. (ed. M. Pessarakly), Boca Raton, CRC Press, Taylor and Frances Group, FL. USA, 2005, 827–843.

[5] Yu S.-G., L. Björn. J. Photochem. Photobiol. B, **34**, 1996, No 1, 35–38.

[6] Hideg E., L. Sass, R. Barbato, I. Vass. Phosysnth. Res., **38**, 1993, No 3, 455–462.

[7] Jordan B. R. Adv. Bot. Res. (ed. J. A. Callow), Academic Press, Harcourt Brace Company, Publishers London, San Diego, NY, Boston, Sydney, Tokyo, Toronto, **22**, 1996, 97–162.

[8] Galatro A., M. Simontacchi, S. Puntarulo. Physiol. Plant., **113**, 2001, No 4, 564–570.

[9] Costa H., S. M. Gallego, M. L. Tomaro. Plant Sci., **162**, 2002, No 6, 939–945.

[10] Koornneef M., C. Alonso-Blanco, D. Vreugdenhil. Annu. Rev. Plant Biol., **55**, 2004, 141–172.

[11] Hannah M., D. Wiese, S. Freund, O. Fiehn, A. G. Heyer, D. K. Hincha. Plant Physiol., **142**, 2006, No 1, 98–112.

[12] Luquez V. M. C., Y. Sasal, M. Medrano, M. I. Martín, M. Mujica, Y. Y. Guiamet. J. Ex. Bot., **57**, 2006, No 6, 1363–1372.

[13] Somerville C. R., S. C. Somerville, W. L. Ogren. Plant Sci. Lett., **21**, 1981, No 1, 89–96.

[14] Zeinalov Y. Bulg. J. Plant Physiol., **28**, 2002, Nos 3–4, 57–67.

[15] Popova A. V., M. Velitchkova, Y. Zanev. Z. Naturforsch., **62c**, 2007, Nos 3–4, 253–260.

[16] Kok B., B. Forbush, M. McGloin. Photochem. Photobiol., **11**, 1970, No 6, 457–475.

[17] Velichkova M., A. Popova. Bioelectrochemistry, **67**, 2005, No 1, 81–90.

[18] Andrizhiyevskaya E. G., A. Chojnicka, J. A. Bautista, B. A. Diner, R. van Grondelle, J. P. Dekker. Photosynth. Res., **84**, 2005, Nos 1–3, 173–180.

[19] Krause G. H., E. Weis. Annu. Rev. Plant Physiol. Plant Mol. Biol., **42**, 1991, 313–349.

[20] Zeinalov Y. In: Handbook of Photosynthesis, 2nd ed. (ed. M. Pessarakly), Boca Raton, CRC Press, Taylor and Frances Group, FL. USA, 2005, 3–19.

Institute of Biophysics and Biomedical Engineering
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 21
1113 Sofia, Bulgaria
e-mail: popova@bio21.bas.bg