

## Oxidative metabolism and antioxidant capacity associated to UV radiation effects in photosynthetic organisms

Andrea Galatro, Maria Belen Aguiar, Gabriela Malanga, and Susana Puntarulo\*

Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, Buenos Aires, Argentina

### ABSTRACT

The stratospheric ozone layer which attenuates solar ultraviolet-B (UV-B) irradiation (290-320 nm) is being depleted by pollutants such as chlorofluorocarbons. It has been postulated that if as a result of ozone loss, UV-B flux at the surface of the earth increases, negative impacts on biological organisms will be inevitable since UV-B radiation causes a multitude of physiological and biochemical changes in photosynthetic organisms. Among other parameters, photosynthesis is impaired, pigment composition is altered, and the expression of the genes which encode for antioxidants are induced. Ultraviolet light has been shown to be very effective in inducing lipid peroxidation of biological membranes, polyunsaturated fatty acids and phospholipid liposomes. It has been also reported that UV-B can destroy the natural liposoluble antioxidants and promote the formation of lipid peroxidation products. The photosynthetic pigments are affected and consequently the production of energy and reduction equivalents decrease, which in turn hampers CO<sub>2</sub> incorporation into organic material. The pigments of the photosynthetic apparatus are affected by solar or artificial UV radiation. The carotenoids, which operate as protective pigments against excessive irradiation,

are bleached and eventually the chlorophyll, vital for photosynthetic energy transformation, is destroyed. In this complex scenario, the mechanisms of biological effects of near UV appear to involve endogenous photosensitization and formation of reactive oxygen species (ROS). The aim of this work is to briefly summarize and update the data on the stress response of photosynthetic cells (both, algae and plants) after exposure to UV-B radiation, comparatively analyzing the effects on the rate of growth, chlorophyll content and chloroplast function described by our laboratory. The profile of the content of lipid-soluble and water-soluble antioxidants is described and analyzed in a general frame to search for adaptive responses.

**KEYWORDS:** UV-B, reactive oxygen species, lipid soluble antioxidants, water soluble antioxidants

### 1. INTRODUCTION

Like all living organisms, photosynthetic organisms sense and respond to UV radiation, both the wavelengths present in sunlight (UV-A and UV-B) and the wavelengths below 290 nm (UV-C). All types of UV radiation are known to damage various plant processes [1]. In spite of the fact that UV-C radiation is not present in sunlight, it has been postulated that the decrease of the stratospheric ozone layer will eventually allow UV-C radiation to reach the earth surface [2]. UV-B and UV-C radiation could have different biological effects because they could be absorbed by different cell components [3]. Panagopoulos *et al.* [4] stated that in general the pattern of response to

---

\*Corresponding author: Dr. Susana Puntarulo,  
Fisicoquímica-PRALIB,  
Facultad de Farmacia y Bioquímica, Junín 956,  
C1113AAD Buenos Aires, Argentina.  
susanap@ffyb.uba.ar

UV-B and UV-C was similar on photosynthesis (except for a stronger effect of UV-C). However, other authors suggest that different photosynthetic reaction centers were inactivated after UV-B and UV-C irradiation [5], and that UV-C and UV-B have opposite effects on carotenoids [6, 2]; and anthocyanin content [7].

The destructive action of UV irradiation results from both, direct and indirect mechanisms involving endogenous sensitizers and the generation of active oxygen species (ROS) [8]. Primary radicals formed as a result of UV irradiation lead to formation of lipid radicals, which react with O<sub>2</sub> to produce lipid peroxy radicals. This sequence can, however, be broken by antioxidants, such as ascorbate (AH<sup>-</sup>) and  $\alpha$ -tocopherol ( $\alpha$ -T). There has been much speculation about the mechanisms that photosynthetic organisms use to perceive and respond to increased UV radiation. Even though a variety of changes in plant normal physiology are either directly or indirectly a consequence of the damaging effects of the energetic radiation, others such as the increases in phenolic compounds accumulation and DNA repair, have been postulated to represent adaptive responses to UV radiation [9, 10, 11]. Perhaps the most general response to UV-B radiation is the activation of the flavonoid biosynthetic pathway, as it was reported in 14 plant species tested [6, 12]. Flavonoids and anthocyanins absorb UV radiation, and generally accumulate in the epidermis, where they could keep UV radiation from reaching photosynthetic tissues [13]. The epidermis blocks transmittance of 95 to 99% of incoming UV radiation [14], and the induction of flavonoids in rye seedlings can prevent UV-B induced damage to photosynthesis [10].

The aim of this work is to briefly summarize and update the data on the oxidative stress response of photosynthetic cells (both, algae and plants) after exposure to UV radiation, comparatively analyzing the effects on the rate of growth, chlorophyll content and chloroplast function. The profile of the content of both lipid-soluble ( $\alpha$ -T,  $\beta$ -Carotene) and water-soluble antioxidants (AH<sup>-</sup>) is described and analyzed in a general frame to search for adaptive responses.

## 2. Oxidative effects of UV on algae and phytoplankton

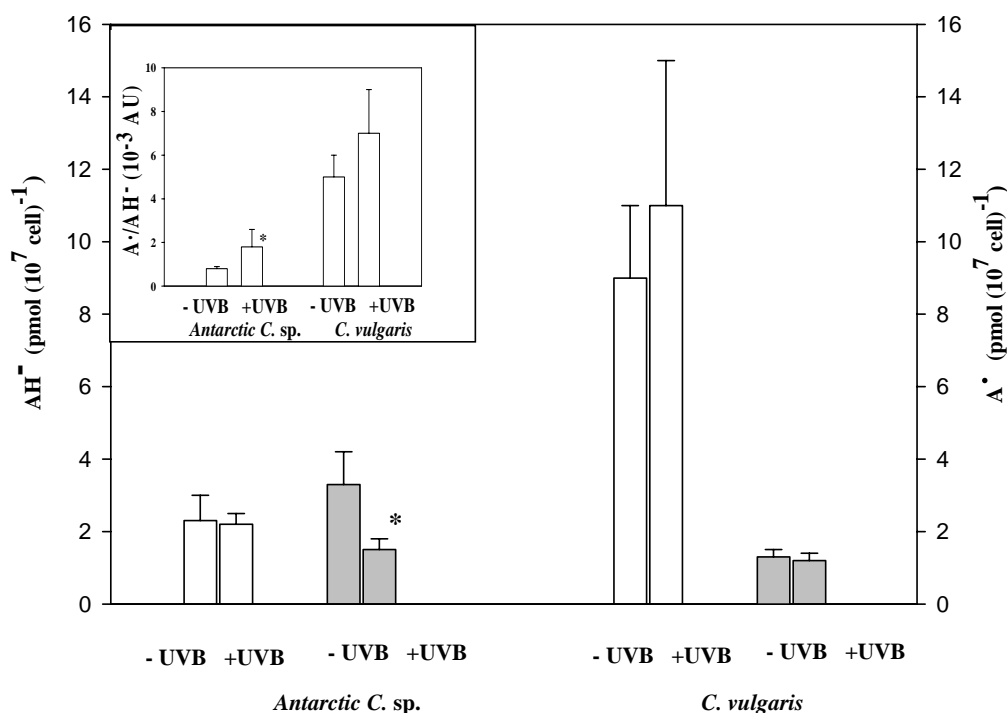
### 2.1. UV-B effects on fresh water microalgae

The effect of UV-B radiation on Antarctic phytoplankton has become an ecological issue as a result of annual springtime ozone depletion over the South Polar region. Even though there are difficulties in relating laboratory UV studies to field studies in marine and freshwater ecosystems, data from laboratory controlled systems could be important for understanding the different sensitivity of photosynthetic organisms to a deepening ozone hole [15]. Aquatic organisms contain a variety of antioxidants including water-soluble compounds (i.e. AH<sup>-</sup>). Ascorbic acid has low redox potential, which allows it to donate one single electron to almost any free radical occurring in a biological system or to reduce oxidized biological radical scavengers, such as  $\alpha$ -T [16]. The ascorbyl radical (A<sup>•</sup>) is the intermediate in the oxidation of AH<sup>-</sup> to dehydroascorbate (DHA) [17]. It has an unpaired electron in a highly delocalized  $\pi$ -system, giving it stability as the “terminal small-molecule antioxidant” [18]. The A<sup>•</sup>/AH<sup>-</sup> ratio was established as an oxidative stress index by measuring A<sup>•</sup> and AH<sup>-</sup> content, in different systems and conditions. For A<sup>•</sup> measurements Electron Paramagnetic Resonance (EPR) has been used [19]. AH<sup>-</sup> content was measured by reverse phase HPLC with electrochemical detection [20]. Estevez *et al.* [21] reported data from a study on oxidative conditions mediated by UV-B exposure employing stock cultures of freshwater Antarctic *Chlorella* sp. collected from one of the two permanent lakes sited at the base of Three Brothers Hill at the Potter Peninsula, King George Island, South Shetland Islands (62°14'S-58°40'W) (Scientific Base Tte. Jubany), and previously Malanga and Puntarulo [15] studied the oxidative response of stock cultures of *Chlorella vulgaris*. The lower growth rate measured in Antarctic *C. sp.* (0.36±0.06 day<sup>-1</sup>) [21], as compared to that previously reported in *C. vulgaris* (0.59±0.07 day<sup>-1</sup>) [21] at their respective optimal temperature of development (2 and 25°C, respectively), might suggest that different oxidative stress conditions could be developed in those organisms. Thus, the A<sup>•</sup>/AH<sup>-</sup>

ratio should be lower in Antarctic *C. sp.* as compared to *C. vulgaris* cells. The data shown in Figure 1 stress this hypothesis since the  $A^{\bullet}/AH^{\bullet}$  ratio for Antarctic *C. sp.* in log phase of development ( $(0.8 \pm 0.2) \cdot 10^{-3}$  AU) was significantly lower than the ratio previously reported for *C. vulgaris* cells,  $((5 \pm 1) \cdot 10^{-3}$  AU) [22]. The oxidative stress conditions developed by UV-B exposure did not alter the content of  $A^{\bullet}$ , but decreased significantly  $AH^{\bullet}$  content in the Antarctic *C. sp.* cells (Figure 1). In *C. vulgaris* cells exposed to UV-B no significant alterations were detected neither in the content of  $A^{\bullet}$  nor in content of  $AH^{\bullet}$  (Figure 1). The  $A^{\bullet}/AH^{\bullet}$  ratio reflected the different behavior of these species, since a significant increase was observed after UV-B exposure in Antarctic *C. sp.* cells and no changes were detected in *C. vulgaris* cells. These results suggest that the effects of environmental oxidative stress are different between species, such as Antarctic *C. sp.* and *C. vulgaris*, and these differences could be an

important factor to be considered in the study of the harmful effects on ecosystems in relation to radiation UV-B in zones where a deepening ozone hole was registered.

Accordingly, a significant decrease in the content of lipid soluble antioxidants was observed in Antarctic *C. sp.* cells and no changes were detected in *C. vulgaris* cells (Table 1). These observations strongly suggest that oxidative conditions were also associated to a decrease on cellular antioxidant capacity. A drastic increase in the use of non-enzymatic antioxidants by the excess of ROS generated by UV-B exposure in the cells not naturally exposed to high UV-B doses could be responsible for the effect. Meanwhile *C. vulgaris* cells did not show this decrease. These comparative results would have a great relevance in ecological terms, since they are a clear example that UV-B effects are species-specific and that adaptation to the natural environment is a critical point to biological survival.



**Figure 1.** UV-B effect on  $A^{\bullet}$  (□) and  $AH^{\bullet}$  content (■) in Antarctic *C. sp.* and *C. vulgaris* in log phase. Insert: UV-B effect of on  $A^{\bullet}/AH^{\bullet}$  ratio (□) in the culture of the algae in log phase. Data are expressed as means  $\pm$  SEM of 4 to 6 independent experiments.

\*significantly different at  $p \leq 0.05$  from the unirradiated cells. ANOVA.

Taken and modified from [21].

**Table 1.** UV-B effect on the content of lipid antioxidants in *Antarctic C. sp.* and *C. vulgaris* in log and stationary phase.

UV-B irradiation (kJ m <sup>-2</sup> )	<i>Antarctic C. sp.</i>		<i>C. vulgaris</i>	
	$\alpha$ -T (pmol (10 <sup>4</sup> cells) <sup>-1</sup> )	$\beta$ -Carotene (pmol (10 <sup>4</sup> cells) <sup>-1</sup> )	$\alpha$ -T (pmol (10 <sup>4</sup> cells) <sup>-1</sup> )	$\beta$ -Carotene (pmol (10 <sup>4</sup> cells) <sup>-1</sup> )
0	0.65±0.05	0.21±0.05	3.4±0.6	0.17±0.02
30	0.034±0.005*	0.08±0.02*	4.6±0.4	0.54±0.06

Measurements for *Antarctic C. sp.* indicated as log phase were performed on day 15<sup>th</sup>. Measurements for *C. vulgaris* indicated as log phase were performed on day 12<sup>th</sup>. Data are expressed as means  $\pm$  SEM of 4 to 6 independent experiments.

\*significantly different, at  $p \leq 0.05$ , from the unirradiated cells in the same phase. ANOVA. Taken and modified from [21].

**Table 2.** Natural effect of UV radiation *in situ* on an Antarctic marine phytoplankton community.

	PAR+UV-A	PAR+UV-A+UV-B
Chlorophyll-a (mg m <sup>-3</sup> )	551 $\pm$ 11	473 $\pm$ 35*
DCF-DA (AU min <sup>-1</sup> (mg prot) <sup>-1</sup> )	362 $\pm$ 53	297 $\pm$ 32*
TBARS (pmol (mg prot) <sup>-1</sup> )	273 $\pm$ 18*	198 $\pm$ 9*

Data are expressed as means  $\pm$  SEM of 3 independent experiments.

\*significantly different, at  $p \leq 0.05$ , from the PAR+UV-A irradiated cells. ANOVA.

UV-B Doses: 10.3 kJ m<sup>-2</sup>, Time of exposure: 3 h

## 2.2. UV-B effects on marine microalgae

The rate of carbon assimilation was widely considered as an index of UV-B effects on the phytoplankton, and many authors estimated the inhibition of the production in marine Antarctic water phytoplankton from 60 to 6.4% by this index [23, 24]. However, this inhibiting effect was only verified at depths of 10-25 m. Gala and Gies [25] reported a small inhibition of the carbon assimilation by UV-B radiation in American Lakes that was attributed to an increase in the tolerance of the community to UV-B, probably due to photo-adaptation and/or changes in the species composition.

To test the hypothesis that intact marine communities of phytoplankton are sensitive to the exposure to natural UV-B, a set of *in situ* experiments were carried out at Potter Cove (South Shetland Islands, Antarctica, 62°14'S, 58°38'W) during the summer

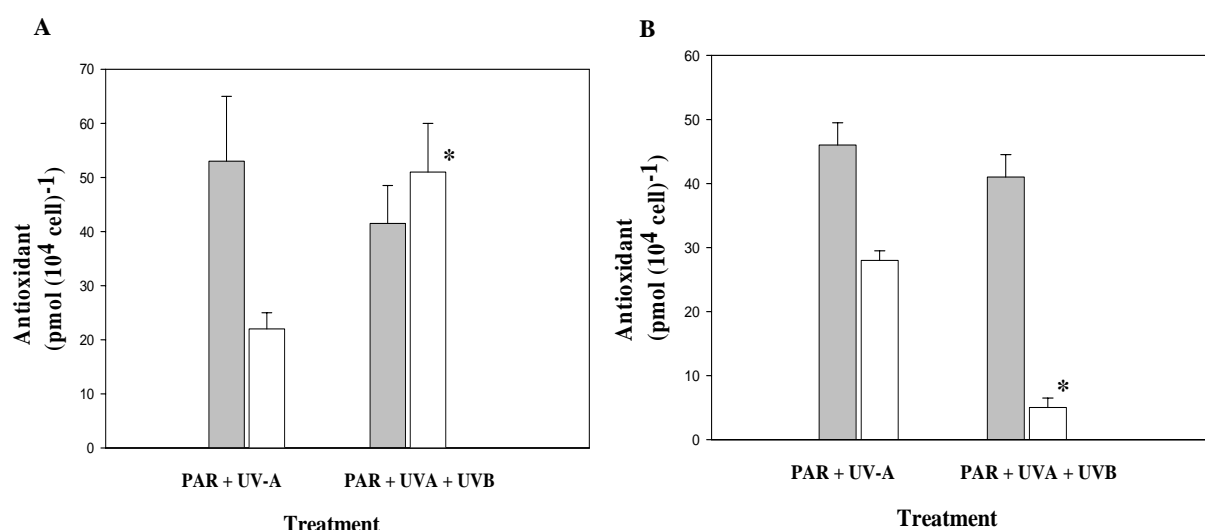
(January). Surface water samples were collected with a five-liter Niskin bottle and maintained in the laboratory at 2°C. Cultures were grown at 210  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> Photosynthetically Active Radiation (PAR, 400-700 nm), and exposed to fluorescent tubes on 15:9 h light:dark periods at 3°C. Prior to the experiments and once exponential growth was reached, aliquots of the cultures were inoculated into a series of 1000 ml vessels and exposed to two natural irradiance treatments: PAR + UV-A and PAR + UV-A + UV-B, in an outdoor water bath with flowing seawater to maintain the temperature between 1-2°C during 3 h. Data in Table 2 show a decrease in growth, assessed as chlorophyll-a content, without a significant increase in the tested parameters (oxidation of the fluorescent probe 2',7' dichlorofluorescein diacetate (DCFH-DA), generation of 2-thiobarbituric acid reactive substances (TBARS)) related to oxidative stress and damage after exposure to UV-B. One of the

proposed mechanisms to explain the phytoplankton resistance to the damage exerted by UV-B, is based on the activity of enzymatic and non-enzymatic antioxidant activity [26, 27]. Many studies demonstrate that over-production of ROS is induced by UV-B radiation in marine algae [28, 29], and there is also significant evidence showing that algae exposed to UV-B stress tends to increase the activities of ROS scavenging enzymes [30-37]. Tian and Yu [38] showed that enhanced UV-B radiation caused ultrastructural changes of *Dunaliella salina*, and induced different responses of antioxidant systems and levels of mycosporine-like amino acids (MAAs) increased at the beginning and subsequently decreased. Moreover, experiments with the diatom, *Thalassiosira oceanica*, showed that the UV radiation and nitrate limitation change the production of biogenic sulfur compound. From the algae community described in Table 2, two species were isolated and cultured, the Antarctic diatom *Thalassiosira* sp. and a phytoflagellate. The antioxidant and photo-protective responses after exposure to surface solar radiation with UV-B under culture conditions during 3 h was studied (Figure 2). The relative contribution to the total protection capacity of  $\alpha$ -T and  $\beta$ -Carotene was analyzed. The Antarctic diatom *Thalassiosira* sp. response to the exposure was a significant

increase in the content of  $\beta$ -Carotene with no alteration in the  $\alpha$ -T content, meanwhile the phytoflagellate cultures showed a significant decrease in the content of  $\beta$ -Carotene with no alteration in the  $\alpha$ -T content, even though both species were isolated from the same marine community. These data suggest that species-specificity is a key factor to be considered when analyzing ecological alterations by UV-B exposure. Recently, Hernando *et al.* [37] postulated that photo-protection against UV-induced damage is characterized by a short-term (3 days) consumption of  $\alpha$ -T and a longer-term (6 days) synthesis of MAAs in the Antarctic diatom *Thalassiosira* sp. UV-B damage/repair ratio during long term exposure, involves the combined action of several endogenous factors within the cell, with MAAs synthesis being the most effective factor related to photo-protection.

### 3. UV effect on terrestrial plants

Despite the uncertainty of long-term predictions, it is estimated an UV-B increase of 5-10% over temperate latitudes within the next 15 years [38]. Thus, it is likely that terrestrial plants will have to deal to enhanced UV-B levels in the next decades, what lead to a significant research effort to better understand the acclimation strategies that could



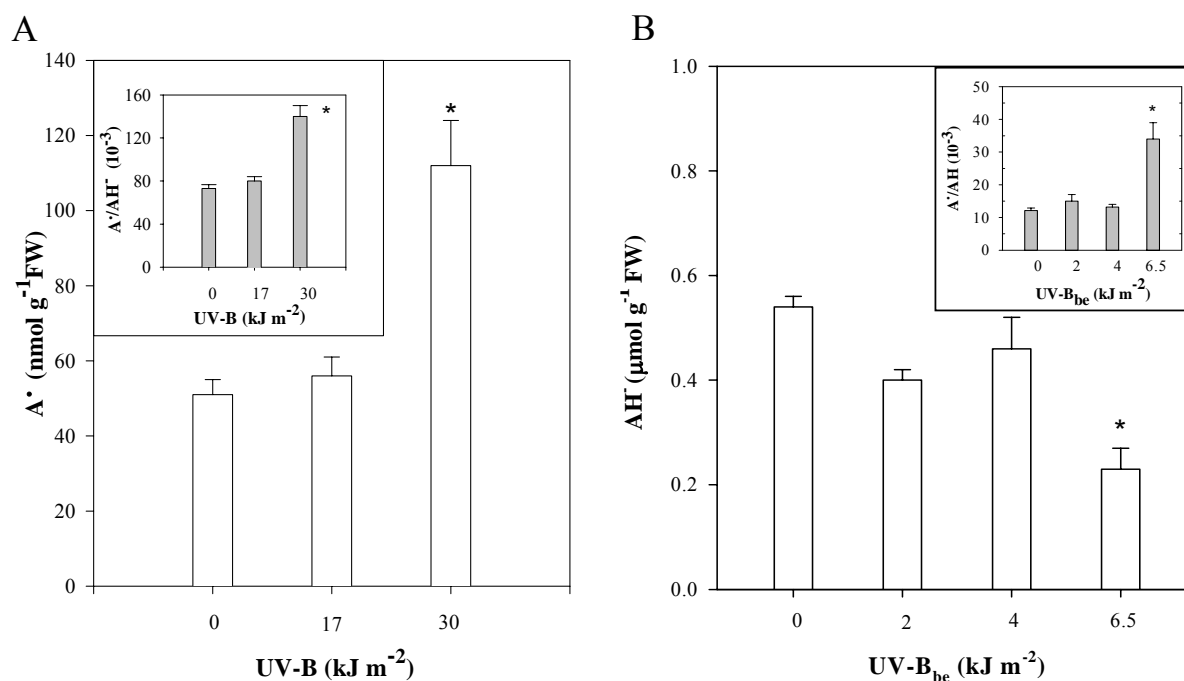
**Figure 2.** UV radiation natural effect *in situ* on antioxidant lipid soluble content in marine phytoplankton. **A.** The content of  $\alpha$ -T (■) and  $\beta$ -Carotene (□) in the Antarctic phytoflagellate; **B.** The content of  $\alpha$ -T (■) and  $\beta$ -Carotene (□) in the Antarctic *Thalassiosira* sp.

help photosynthetic organisms to cope with its harmful effects [39]. The damaging effectiveness of UV-B varies both among species and cultivars of a given species. Sensitive plants often exhibit reduced growth, photosynthetic activity and flowering. Photosynthetic activity may be reduced by direct effects on photosynthetic enzymes, metabolic pathways or indirectly through effects on photosynthetic pigments or stomatal function [40]. ROS are involved in the responses of plants to UV-B, as signaling molecule and as damaging agents [41]. Increased antioxidant activity [42],  $A^\bullet$  content [43–45], long-lived chlorophyll radicals [46] as well as oxygen-, carbon-, and nitrogen-centered free radicals [44, 45, 47] were detected by EPR spectroscopy in a variety of plants upon UV-B exposure. Regarding the oxidative stress conditions in plants due to UV-B exposure, the recorded effects on leaves has been mimicked by free radical generators and prevented by antioxidant feeding [48], however action mechanisms are not identical for all the species. DCF-DA oxidation was not significantly different in control and treated soybean (*Glycine max*) leaves at 24 h after exposure to  $30 \text{ kJ m}^{-2}$  UV-B [49]. Moreover, no significant differences in the content of lipid radicals were detected among treatments not only in soybean [47], but also in *G. magellanica* (a perennial herb native plant species from Tierra del Fuego in southernmost Patagonia) leaves after UV-B irradiation [45]. However, high levels of UV-B were shown to increase peroxidation [50–54] under different experimental conditions.

The  $A^\bullet/AH^-$  ratio was comparatively employed to evaluate the UV-B effects in leaves from both soybean and *G. magellanica* plants. The exposure of soybean leaves to  $30 \text{ kJ m}^{-2}$  UV-B increased the content of  $A^\bullet$  (100 %) as early as 24 h post-irradiation with no change in  $AH^-$  content [49]. Thus, either  $A^\bullet$  content by itself or the  $A^\bullet/AH^-$  ratio could be employed successfully as an indicator of oxidative stress in the hydrophilic medium (Figure 3 A). It was proposed that the increase in the steady state concentration of  $A^\bullet$  could be due to an increased production of ROS or to a decrease in the capacity of  $AH^-$  regeneration [55]. However, when  $AH^-$  content is altered, the  $A^\bullet/AH^-$  ratio is the appropriate

index to estimate early oxidative conditions in the hydrophilic medium. *G. magellanica* was exposed to UV-B (biologically effective UV doses,  $UV-B_{be}$ ) in a greenhouse experiment, in a range selected to mimic natural levels in Tierra del Fuego [45]. Under these conditions, the content of  $A^\bullet$  was not significantly affected upon the initial 10 days of exposure. However, the  $AH^-$  content was significantly decreased after 4 days of treatment, and accordingly the  $A^\bullet/AH^-$  ratio significantly increased in leaves exposed to  $6.5 \text{ kJ m}^{-2} \text{ d}^{-1}$   $UV-B_{be}$ , as compared to control plants (Figure 3B insert). These results allowed us to show that different pathways could lead to oxidative stress in the hydrophilic milieu. This study with *G. magellanica* also showed that on day 10,  $AH^-$  content increased in leaves exposed to UV-B, and  $A^\bullet/AH^-$  ratio decreased to control levels. These observations suggest a key role for the regulation of the  $AH^-$  pool in the acclimation mechanisms to limit the potential oxidative damage caused by UV-B exposure. In agreement with these observations, Gao and Zhang [56], reported that short term increases in UV-B induced an increase in  $H_2O_2$  content and the production of TBARS in  $AH^-$ -deficient *vct1* mutants of *Arabidopsis thaliana* associated to an increased ratio dehydroascorbate/ $AH^-$ , and a reduced ratio GSH/total glutathione as compared to the wild type plants.

Although Carletti *et al.* [57] evidenced a decrease in  $\alpha$ -T and  $\gamma$ -T content, and an increase in  $AH^-$  content in *Zea Mays* L. exposed to UV-B ( $8.35 \text{ kJ m}^{-2} \text{ d}^{-1}$ ), antioxidant levels were not affected after UV-B radiation in leaves and cotyledons from soybean irradiated with a single dose of  $30 \text{ kJ m}^{-2}$  UV-B, after 7 days of growth (Table 3). However, it is interesting to point out that cotyledons exposed to a single dose of  $30 \text{ kJ m}^{-2}$  UV-B+UV-C radiation showed a significant increase in the content of  $\alpha$ -T (2.3 fold) and  $AH^-$  (1.2-fold) indicating that some mechanisms could be operative under more stressful situations such as UV-C exposure [58]. Soybean cotyledons exposed to UV-C radiation accumulated a colored pigment (apigeninidin) in the epidermis as early as 15 h after receiving the UV-C irradiation, with a maximum 24 h post-irradiation. Pigmentation was observed in areas of the epidermis directly exposed to the UV-C source [59]. The presence of



**Figure 3. A.** UV-B effect on A• content in soybean leaves. Insert: A•/AH• ratio in soybean leaves exposed to a single dose of UV-B. Plants were grown for 7 days and were exposed to a single dose of UV-B. **B.** UV-B effect on AH• content in *G. magellanica* leaves. Plants were exposed to UV-B<sub>be</sub> doses daily. The different daily doses were obtained by exposing the plants during 3, 6 or 10 h to the UV-B radiation emitted by the lamps. Insert: A•/AH• ratio in *G. magellanica* leaves. \*significantly different at  $p \leq 0.05$  from the unirradiated leaves. ANOVA. Taken and modified from [45, 49].

**Table 3.** UV-B effect on the content of antioxidants in soybean.

	Antioxidants					
	$\alpha$ -T (nmol g <sup>-1</sup> FW)		$\beta$ -Carotene (nmol g <sup>-1</sup> FW)		Ascorbic acid (μmol g <sup>-1</sup> FW)	
	- UV	+ UV	-UV	+ UV	- UV	+ UV
Leaves	10±1	8±1	2.7±0.4	3.0±0.4	0.7±0.1	0.8±0.2
Cotyledons	6±1	5±1	5.8±0.9	6.5±0.9	0.9±0.2	1.0±0.1

Soybean plants were irradiated with single dose of 30 kJ m<sup>-2</sup> UV-B, after 7 days of growing and determinations were done 24 h after treatment in homogenates from leaves, and in homogenates from the surface layers of cotyledons exposed to UV-B radiation.

Taken and modified from [49] and [58].

apigeninidin in soybean was an interesting finding since aglycones are not normally found in living tissues. EPR techniques were applied to study the antioxidant capacity of apigeninidin that showed ability for scavenging A• and lipid radicals [59]. Although protection from UV-C radiation is not

currently biologically important, the observation that in soybean cotyledons there are metabolic pathways that could be triggered by UV-C radiation seems as relevant mechanistic information. Since adaptation to photo-oxidative stress is multifactorial, it may be possible that UV-C irradiation activates

molecular signals in common with more than one metabolic pathway. In this sense, soybean cotyledons from plants growing in a nutrient solution of Steingberg [60] supplemented with  $\text{CuSO}_4$  (50  $\mu\text{M}$ ) evidenced the appearance of a pigment on the surface of the cotyledon that could be related to apigeninidin. Moreover, since it was reported that flavonoid aglycones increased antioxidant capacity of blood plasma [61] and soybean products are widely consumed in Western and Asian diets for health benefits, it could be of potential interest to use this cellular response to develop strategies to improve antioxidant capacity of soybean before the utilization of this products in the food and cosmetic industries.

#### 4. UV effect on chloroplasts

The chloroplasts with their strong photo-oxidative potential are the most likely organelle to be affected by a significant production of ROS [62], since their membranes, rich in polyunsaturated fatty acids [63], are particularly vulnerable to oxidative damage. However, data included in Table 4, showed that both  $\text{O}_2$  production by chloroplasts and chlorophyll-a content were significantly affected

by UV-B in algae, but not in soybean leaves. Lipid peroxidation, assessed by TBARS content, did not show any significant difference after irradiation in neither of the isolated organelles. However, chloroplasts from *Oryza sativa* L. plants showed an increase of 28% in the content of malondialdehyde (MDA) 24 h after UV-B exposure (2.975  $\text{kJ m}^{-2} \text{d}^{-1}$  UV-B<sub>be</sub> for 7 days) [39], and chloroplasts of cluterebean (*Cyamopsis tetragonoloba*) also showed an increase of MDA levels of 33% in response to irradiation (fluence rate of 60  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 1 h daily for 10 days) [64]. Thus, a more sensitive and specific technique, such as EPR, was employed to analyze the UV-B effect on chloroplast from algae [65] and soybean [44]. This methodology allowed the detection of an increase of 3-fold (algae chloroplasts) and 2-fold (soybean chloroplasts) in the content of lipid radicals after UV-B exposure (Table 4). These results were in agreement with previous reports from Hideg and Vass [66] in thylakoid membranes isolated from leaves of *Vicia faba* plants.

The mechanisms triggered to exert the antioxidant capacity required to deal with the effects of UV-B increases proved to depend on the photosynthetic

**Table 4.** Effect of UV-B irradiation on chloroplasts from *C. vulgaris* and soybean leaves.

	Algae		Soybean	
	- UV-B	+ UV-B	- UV-B	+ UV-B
$\text{O}_2$ production <sup>1</sup>	4.7 $\pm$ 0.5	1.3 $\pm$ 0.3*	106 $\pm$ 10	101 $\pm$ 9
Chlorophyll-a content <sup>2</sup>	1.3 $\pm$ 0.3	0.44 $\pm$ 0.04*	43 $\pm$ 5	44 $\pm$ 8
TBARS content <sup>3</sup>	3 $\pm$ 1	2.9 $\pm$ 0.3	0.61 $\pm$ 0.07	0.98 $\pm$ 0.09
Lipid radical content <sup>4</sup>	0.37 $\pm$ 0.12	1.39 $\pm$ 0.32*	9 $\pm$ 1	18 $\pm$ 2*
$\alpha\text{-T}$ <sup>5</sup>	3.1 $\pm$ 0.4	14 $\pm$ 5*	8 $\pm$ 1	7 $\pm$ 1
$\beta\text{-Carotene}$ <sup>6</sup>	13 $\pm$ 4	12 $\pm$ 3	2.8 $\pm$ 0.3	2.6 $\pm$ 0.5
AH <sup>7</sup>	183 $\pm$ 41	145 $\pm$ 33	2.3 $\pm$ 0.4	5 $\pm$ 1*

Algae cultures were irradiated (42.6  $\text{kJ m}^{-2}$ ) at day 0 of development and measurements were done in stationary phase (day 18). Soybean leaves were irradiated (60  $\text{kJ m}^{-2} \text{day}^{-1}$ ) for 4 days and measurements were done 24 h after the last irradiation.

<sup>1</sup>nmol  $\text{O}_2$  ( $10^7$  cells)<sup>-1</sup> min<sup>-1</sup> for chloroplasts from algae, and  $\mu\text{mol O}_2$  (mg chlorophyll)<sup>-1</sup> h<sup>-1</sup> from soybean leaves.

<sup>2</sup> $\mu\text{g}$  ( $10^7$  cells)<sup>-1</sup> for chloroplasts from algae, and  $\mu\text{g}$  (mg protein)<sup>-1</sup> from soybean leaves.

<sup>3</sup>nmol ( $10^9$  cells)<sup>-1</sup> for chloroplasts from algae, and nmol (mg protein)<sup>-1</sup> from soybean leaves.

<sup>4</sup>pmol ( $10^7$  cells)<sup>-1</sup> for chloroplasts from algae, and pmol (mg protein)<sup>-1</sup> from soybean leaves.

<sup>5,6,7</sup>pmol ( $10^7$  cells)<sup>-1</sup> for chloroplasts from algae, and nmol (mg protein)<sup>-1</sup> from soybean leaves.

Data are expressed as means  $\pm$  E.S.M. of 4 to 6 independent experiments.

\*significantly different at  $p < 0.05$ , from control values (un-irradiated tissues). ANOVA.

Taken and modified from [44] and [65].



organism studied probably according to the biological mechanism of signal transduction involved. Data shown in Table 4 describe that in soybean chloroplasts the content of neither  $\alpha$ -T nor  $\beta$ -Carotene was affected by the irradiation suggesting that lipid soluble antioxidants were not increased as an adaptive response to UV-B irradiation, leading to an enhancement of lipid radical generation and oxidized proteins [44]. On the other hand, chloroplasts from *C. vulgaris* increased  $\alpha$ -T content (4.5-fold), however this response was not enough to adequately control the lipid oxidation in the lipid phase since a drastic increase in lipid radical content was observed after UV-B exposure. Regarding the hydrophilic milieu, also a difference in the response of the chloroplasts isolated from algae and soybean was observed. The content of  $AH^\cdot$  in chloroplasts from *C. vulgaris* cells did not change after UV-B exposure (Table 4), but the content of the water soluble antioxidant was increased by 117% in chloroplasts isolated from soybean leaves (Table 4), suggesting differential pathways triggered also at this level.

To further characterize the effect of UV-B on isolated chloroplasts from soybean leaves the  $A^\cdot$  content was recorded. A strong EPR doublet with the spectral features ( $a_H=1.88$  G,  $g=2.0054$ ) of the  $A^\cdot$  was detected; however no differences in the content of  $A^\cdot$  were observed after the exposure to UV-B. Thus,  $A^\cdot/AH^\cdot$  ratio was not increased (even was decreased) under these experimental conditions suggesting that in isolated chloroplasts either an active exchange between the chloroplasts and the cytosol was established [67], or the mechanisms to reconvert  $A^\cdot$  to  $AH^\cdot$  remained efficient. Moreover, the increase in  $AH^\cdot$  was also accompanied by an increase in total thiol content (20.8%), in chloroplasts from leaves exposed to  $60 \text{ kJ m}^{-2} \text{ day}^{-1}$  UV-B, highlighting the importance of the  $AH^\cdot/A^\cdot$  and glutathione/GS $^\cdot$  redox couples which are proposed to be linked [68]. These observations are consistent with the lack of effect of UV-B exposure on the functionality of the chloroplasts (Table 4), despite the slight decrease in the Hill reaction by 19% after *in vivo* irradiation with  $60 \text{ kJ m}^{-2} \text{ day}^{-1}$  UV-B [44].

Malanga *et al.* [65] showed results employing dihydrorhodamine 123 (DHR) conversion to R123, as a sensitive method to detect the formation of

peroxides (such as  $H_2O_2$ ). In chloroplasts from algae irradiated with  $42.6 \text{ kJ m}^{-2}$  UV-B, DHR oxidation was increased by 103%. This result suggested that generation of hydroperoxides was increased in chloroplasts after UV-B exposure probably due to the lack of the antioxidant response at the hydrophilic media. Moreover, the increase in peroxides (such as  $H_2O_2$ ) were in agreement with the damage observed in the DNA evidenced by the detection of the oxidized DNA base (8-hydroxy-2'-deoxyguanosine) in the chloroplasts from *C. vulgaris* [65]. Both the  $O_2$  production and chlorophyll-a content were decreased by 72 and 66%, respectively by irradiation of the cells with UV-B in chloroplasts from *C. vulgaris* (Table 4), perhaps reflecting the lack of a strong response in the water soluble milieu of the chloroplasts.

## CONCLUDING REMARKS

The data summarized here strongly emphasize the idea that UV-B exposure triggers a different set of defense pathways in the studied photosynthetic tissues. Moreover, these results support the idea that the UV-B damage/repair balance involves the combined action of several endogenous cellular factors within the cell. Besides the importance of the knowledge of the effect of UV-B exposure in photosynthetic organisms in terms of the consequences of the ozone hole in the South Atlantic, UV-B radiation impacts on the level of a broad range of metabolites, including phenolic, terpenoid and alkaloid compounds that are pharmacologically active and/or nutritionally important [42]. The level of some of these metabolites increase after UV-B exposure, while others decrease, show a transient change, or are differently affected by low and high UV doses. In this context, Jansen *et al.* [42] concluded that the complex pattern of stress-induced changes in plant metabolites need to be studied in more detail to determine impacts on the nutritional and pharmacological characteristics of food products to prove that UV-B acclimated plants could have nutritional benefits. Thus, based on these and future studies in this direction, the benefit of the exposure of plants (such as soybean) or algae, previous to their use as food, could improve their antioxidant content and as a consequence enhance their value as nutrients for humans.

## ACKNOWLEDGMENTS

These studies were supported by grants from the University of Buenos Aires, and CONICET. S. P., G. M., and A. G. are career investigators from CONICET and M. B. A is a fellow from CONICET.

## REFERENCES

1. Stapleton, A. E. 1992, *Plant Cell.*, 4, 1353-1358.
2. Campos, J. L., Figueras, X., Pinol, M. T., Boronat, A., and Tiburcio, A. F. 1991, *Photochem. Photobiol.*, 53, 689-693.
3. Cerdá Olmedo, E., Martín-Rojas, V., and Cubero, B. 1996, *Photochem. Photobiol.*, 64, 547-551.
4. Panagopoulos, I., Bornman, J. F., and Biorn, L. O. 1989, *Physiol. Plant.*, 76, 461-465.
5. Kulandaivelu, G. and Noorudeen, A. M. 1983, *Physiol. Plant.*, 58, 389-394.
6. Tevini, M., Iwanzik, W., and Thoma, U. 1981, *Planta*, 153, 388-394.
7. Hashimoto, T., Scichijo, C., and Yatsushashi, H. 1991, *J. Photochem. Photobiol. B*, 11, 353-363.
8. Foyer, C. H., Lelandais, M., and Kunert, K. J., 1994, *Physiol. Plant.*, 92, 696-717.
9. Li, J., OU-Lee, T. M., Raba, R., Amundson, R., and Last, R. L. 1993, *Plant Cell*, 5, 171-179.
10. Tevini, M., Braun, J., and Fieser, G. 1991, *Photochem. Photobiol.*, 53, 329-333.
11. Lois, R. and Buchanan, B. B. 1994, *Planta*, 194, 504-509.
12. Beggs, C. J. and Wellmann, E. 1985, *Photochem. Photobiol.*, 41, 481-486.
13. Hahlbrock, K. and Scheel, D. 1989, *Ann. Rev. Plant Physiol. Plant Molec. Biol.*, 40, 347-369.
14. Robberecht, R. and Caldwell, M. M. 1978, *Oecologia*, 32, 277-287.
15. Malanga, G. and Puntarulo, S. 1995, *Physiol. Plant.*, 94, 672-679.
16. Vergely, C., Maupoil, V., Clermont, G., Bril, A., and Rochette, L. 2003, *Arch. Biochem. Biophys.*, 420, 209-216.
17. Hubel, C. A., Kagan, V. E., Kosin, E. R., McLaughlin, M., and Roberts, J. M. 1997, *Free Radic. Biol. Med.*, 23, 597-609.
18. Buettner, G. R. 1993, *Arch. Biochem. Biophys.*, 300, 535-543.
19. Giulivi, C. and Cadenas, E. 1993, *FEBS Lett.*, 332, 287-290.
20. Malanga, G., Aguiar, M. B., and Puntarulo, S. 2011, *Book on Oxidative Stress in Aquatic Ecosystems*, Abele, D., Zenteno-Savín, T., and Vázquez-Medina, J. P. (Eds.) Willey-Blackwell, ISBN 9781444335484, in press.
21. Estevez, M. S., Malanga, G., and Puntarulo, S. 2001, *J. Photochem. Photobiol. B: Biol.*, 62, 19-25.
22. Estevez, M. S., Malanga, G., and Puntarulo, S. 2001, *Plant Sci.*, 106, 9-17.
23. Helbling, E. W., Villafañe, V. E., and Holm-Hansen, O. 1994, *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*, Weiler, C. S. and Penhale, P. A. (Eds), American Geophysical Union. Washington, 62, 207-227.
24. Smith, R. C., Prezelin, B. B., Baker, K. S., Bidigare, R. R., Boucher, N. P., Coley, T., Karents, D., MacIntyre, S., Malick, H. A., Menzies, D., Ondrusek, M., Wan, Z., and Waters, K. J. 1992, *Science*, 255, 952-959.
25. Gala, W. R. and Gies, J. P. 1991, *Ecotoxicol. Environ. Safety*, 22, 345-361.
26. Davidson, A. T. 1998, *Mut. Res.*, 422, 119-129.
27. Niyogi, K. K. 1999, *Annu. Res. Plant Physiol. Plant Mol. Biol.*, 50, 333-359.
28. Rijstenbil, J. W. 2002, *J. Plankton Res.*, 24, 1277-1288.
29. Shiu, C.-T. and Lee, T.-M. 2005, *J. Exp. Bot.*, 56, 2851-2865.
30. Huang, J., Tang, X. X., Liu, T., and Li, Y. Q. 2002, *J. Ocean University of Qingdao*, 32(4), 574-578.
31. Rijstenbil, J. W. 2002, *J. Plankton Res.*, 24(12), 1277-1288.
32. Rossa, M. M., de Oliveira, M. C., Okamoto, O. K., Lopes, P. F., and Colepicolo, P. 2002, *J. Appl. Phycol.*, 14(3), 151-157.
33. Shiu, C. T. and Lee, T. M. 2005, *J. Exp. Bot.*, 56(421), 2851-2865.
34. Wang, Y. and Tan, X. X. 2005, *Chin. J. Appl. Ecol.*, 16(8), 1507-1512.
35. Li, L. L., Dong, K. S., and Tang, X. X. 2008, *Environ. Sci.*, 29(10), 2766-2772.
36. Li, L., Zhao, J., and Tang, X. J. 2010, *Environ. Sci.*, 22(5), 716-722.

37. Hernando, M., Malanga, G., Puntarulo, S., and Ferreyra, G. 2011, *Lat. Am. J. Aquat. Res.*, in press.
38. Tian, J. and Yu, J. 2009, *J. Photochem. Photobiol. B: Biol.*, 97, 152-160.
39. Lidon, F. C. and Ramalho, J. C. 2011, *J. Photochem. Photobiol. B: Biol.*, 104, 457-466.
40. Tevini, M. and Teramura, A. H. 2008, *Photochem. Photobiol.*, 50, 479-487.
41. Snrychová, I., Kóss, P. B., and Hideg, E. 2007, *Funct. Plant Biol.*, 34, 1112-1121.
42. Jansen, M. A. K., Hectors, K., O'Brien, N. M., Guisez, Y., and Potters, G. 2008, *Plant Sci. j.plantsci2008.04.010/j.plantsci2008.4.010*.
43. Hideg, E., Mano, J., Ohno, C., and Asada, K. 1997, *Plant Cell Physiol.*, 25, 684-690.
44. Galatro, A., Simontacchi, M., and Puntarulo, S. 2001, *Physiol. Plant.*, 113, 564-570.
45. Giordano, C. V., Galatro, A., Puntarulo, S., and Ballare, C. L. 2004, *Plant Cell Environ.*, 27, 1415-1423.
46. Kumagai, J., Katoh, H., Miyazaki, T., Hidema, J., and Kumagai, T. 1999, *J. Rad. Res.* 40, 303-310.
47. He, J. M., Xu, H., She, X. P., Song, X. G., and Zhao, W. M. 2005, *Functional Plant Biology*, 32, 237-247.
48. Mackerness, A. H., Surplus, S., Jordan, S. L., and Thomas, B. R. 1998, *Photochem. Photobiol.*, 68, 88-96.
49. Kozak, R. G., Malanga, G., Caro, A., and Puntarulo, S. 1997, *Recent Dev. Plant Physiol.*, 233-239.
50. Yannarelli, G. G., Gallego, S. M., and Tomaro, M. L. 2006, *Environ. Exp. Bot.*, 56, 174-181.
51. Hideg, E., Nagy, T., Oberschall, D., Dudits, D., and Vass, I. 2003, *Plant Cell Environ.*, 26, 513-522.
52. Costa, H., Gallego, S. M., and Tomaro, M. L. 2002, *Plant Sci.*, 162, 939-945.
53. Alexieva, V., Sergiev, I., Mapelli, S., and Karanov, E. 2001, *Plant Cell Environ.*, 24, 1337-1344.
54. Xu, C., Natarajan, S., and Sullivan, J. H. 2008, *Environ. Exp. Bot.*, 63, 39-48.
55. Heber, U., Miyake, C., Mano, J., Ohno, C., and Asada, K. 1996, *Plant Cell Physiol.*, 37, 1066-1072.
56. Gao, Q. and Zhang, L. 2008, *J. Plant Physiol.*, 165, 138-148.
57. Carletti, P., Masi, A., Wonisch, A., Grill, D., Tausz, M., and Ferretti, M. 2003, *Environ. Exp. Bot.*, 50, 149-157.
58. Kozak, R. G., Ricco, R., Gurni, A., Boveris, A. D., and Puntarulo, S. 1999, *Can. J. Plant Sci.*, 79, 181-189.
59. Boveris, A. D., Galatro, A., Zambrotta, L., Ricco, R., Gurni, A. A., and Puntarulo, S. 2001, *Phytochemistry*, 58, 1097-1105.
60. Tiffin, L. O. 1966, *Plant Physiol.*, 41, 510-514.
61. Hempel, J., Pforte, H., Raab, B., Engst, W., Bohm, H., and Jacobasch, G. 1999, *Nahrung*, 43, 201-204.
62. Salin, M. L. 1991, *Free Radic. Res. Commun.*, 12-13, 851-858.
63. Chow, W. S., Strid, A., and Anderson, J. M. 1992, *Research in photosynthesis*, Murata, N. (Ed.), Kluwer Academic Publishers, Dordrecht, 361-364.
64. Joshi, P., Gartia, S., Pradhan, M. K., and Biswal, B. 2011, *Plant Sci.*, 181, 90-95.
65. Malanga, G., Calmanovici, G., and Puntarulo, S. 1997, *Physiol. Plant.*, 101, 455-462.
66. Hideg, E. and Vass, I. 1996, *Plant Sci.*, 115, 251-260.
67. Luwe, M. W. F., Takahama, U., and Heber, U. 1993, *Plant Physiol.*, 101, 969-976.
68. Wefers, H. and Sies, H. 1988, *Eur. J. Biochem.*, 174, 353-357.