Egypt. Acad. J. Biolog. Sci.,3(1): 19- 30(2012) **H. Botany**
Email: egyptianacademic@yahoo.com **ISSN 2090-3812**
Received: 19 / 5 /2012 **www.eajbs.eg.net**

HPLC analysis of non-enzymatic antioxidants in *Azolla caroliniana* (Pteridopsida) subjected to UV-B

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ABSTRACT

The ultrastructure of chloroplast and quantified antioxidant compounds such as α-tocopherol, ascorbic acid and beta carotene, semi quantitatively by comparing the peak areas of HPLC chromatograms, were affected considerably in response to exposure of *Azolla caroliniana* to UV-B radiation for 24 and 48 h. The results show that the most striking effect of the enhanced UV-B treatment on the ultrastructure of *A. caroliniana* was observed after 48 h as markedly widened chloroplast area occupied by large amount of plastoglobuli in stressed fronds. Prolonged UV-B exposure of *A. caroliniana* for 48 h resulted in a reduction in peaks area for for α-tocopherol, ascorbic acid and beta-carotene. The results show that changes in the chromatogram of non-enzymatic antioxidants were correlated with the duration of exposure to UV-B radiation. These antioxidant metabolites play a vital role in protecting *A. caroliniana* to a certain limit against UV-B radiation.

Keywords: HPLC- antioxidants- *Azolla caroliniana*- UV-B

INTRODUCTION

Human impact through urban activates and industrial gas emissions such as chlorofluorocarbons and nitrogen oxides in the stratosphere was found to explain the low level of ozone concentration in the last 30 years (Solomon, 2008). Therefore, it still remains interesting to investigate the effects of elevated UV-B radiation on various aspects of plant growth, continuously (Solomon, 2008; Kakani *et al*., 2003 and Wang *et al*., 2006).

Plants have evolved mechanisms to avoid and repair UV radiation oxidative stress damage, and the free radicals caused by UV tend to be involved in the induction of antioxidant defense system. In previous studies, it has been concluded that plant growth and biomass accumulation could result from complex interactions between harmful direct and indirect effects of UV and a series of counteracting repair mechanisms (Lesser *et al*., 1994 and Ibrahim and Mostafa, 2007). It has been shown that the composition of the aquatic fern *Azolla* varies profoundly according to environmental conditions and the species (Lumpkin and Plucknett, 2008; Van Hove, 1987 and Sanginga, N. and Van Hove, 1989).

Reactive oxygen species (ROS) such as superoxide radicals ($O_2^{-\cdot}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) are present in all plants in varying degrees as a result of normal aerobic metabolism. Under biotic or abiotic stress, production and removal of ROS are controlled by an array of enzymatic and non-enzymatic antioxidant mechanisms in plants reducing damage to DNA, protein, and lipids (Foyer *et al*., 1994; Singh *et al*., 2006 and Ibrahim and Bafeel, 2011). Non-enzymatic antioxidant defense systems consist of low molecular mass compounds such as ascorbic acid and glutathione which are active in the aqueous

phase, whereas the lipophilic antioxidants (such as A-tocopherol and ß-carotene) are active in the membrane environment (Asada, 1994).

Alpha-tocopherol is known for its protective effect against lipid peroxidation of biological membranes via peroxyl and alkoxyl radical scavenging (Burton *et al*., 1982). Beta carotene content of *Azolla* is of particular interest since this nutrient is usually required as a supplement in animal feeding as physiological antioxidant and as a precursor of vitamin A, a multipurpose vitamin essential for animal health. Also, other function of ß-carotene is photoreceptive, because it acts as a pigment antenna in the photosynthesis process. Relatively low concentrations of ß-carotene are able to protect plants against oxidative damage which initiated by singlet oxygen (Lowe *et al*., 1999).

Microscopic studies could serve as an additional and sensitive tool in the assessment of plant responses to UV-B. By means of electron microscopy, early stress responses can be observed in the cell structure before the first macroscopic visible symptoms occur (Holopainen *et al*., 1992). Special emphasis was put on chloroplast ultrastructure because alterations in the chloroplast could lead to changes in carbon assimilation and biomass accumulation (Ibrahim and Mostafa, 2007). Furthermore, it is well established that chloroplast structure is altered by UV-B usually before other cell organelles (Holopainen *et al*., 1996).

Azolla, is an aquatic fern native of Asia and Africa. There is a symbiotic relationship between *Azolla* and a nitrogen fixer, the cyanobacteria *Anabaena azollae* which invades certain cavities on the dorsal lobes of the leaves on the ventral surface. It is used as a biofertilizer to crops because of its ability to fix dinitrogen at high rates and low cost.

Our previous study has shown that, the vegetative growth, the photosynthetic pigments in addition to the UV-absorbing compounds such as phenolics, flavonoids and anthocyanins in *Azolla caroliniana* were strongly affected by the duration of exposure to UV-B radiation (Ibrahim and Mostafa, 2007). In this study, changes in reservation non-enzymatic antioxidant metabolites and ultrastructure of chloroplast were investigated in relation to UV damage in the aquatic fern *Azolla caroliniana*.

MATERIALS AND METHODS

Plants and cultivation

Azolla caroliniana Wild (Known as water velvet) was provided by Prof. Weam El-Aggan in year 1982 from Catholic University of louvain, Belgium, it was identified by Prof. Peters G.A., Kettering laboratory Yellow Springs, Ohio 45387. The plants were acclimated in the green-house of the Faculty of Science, Alexandria, in 2500 cm^3 polyethylene vessels which were filled with a nitrogen free, modified [KNO_3 and $Ca(NO_3)_2$ were replaced by KCl and $CaCl_2$, respectively] Hoagland solution (2/5 concentration, pH 5.1). About 5g (fresh mass) of *Azolla* from the stock material were inoculated in each vessel to make a new subculture, and so on. The plants were freed from epiphytic microorganisms by thorough washing with distilled water. The cultures were grown in a growth chamber under 16-h photoperiod at irradiance of 1200 μmol m^{-2} S^{-1} (cool white fluorescent tubes) and (light/dark) temperature of 28-30/20-25 °C (Stock culture). Before being used the plants were surface sterilized with 0.2 % Clorox (El-Aggan, 1982), then thoroughly washed with water.

Treatments

Five grams of *Azolla* plants were transferred to 250 cm^3 vessels containing 2/5 modified Hoagland solution at laboratory conditions for 7d then exposed to UV-B radiation supplied by three UV-emitting tubes (TL12/100W/01, Philips, Holland) positioned 50 cm above leaf level .Rack height, lamp spacing and lamp power were adjusted as described by Ibrahim and Mostafa (2007) to maintain a total daily flux of biologically effective UV-B radiation doses 5.75 KJ $m^{-2}d^{-1}$. Plants were rotated under the lamp banks in an attempt to minimize potential effects resulting from micro-environment variation for 0, 24 and 48 h. Samples were taken for chemical analyses and ultrastructure examination.

Methods

Transverse sections of *Azolla* plants were examined by both light microscope (LM) and transmission electron microscopy (TEM). Small pieces (1 mm^2) of *Azolla caroliniana* with irregular brown patches caused by UV-B and of dark-green fronds (control) were taken for electron microscopy analysis. Fragments of the control and treated fronds were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.8, for 2 h at 0–4°C. After washing with the same buffer, small pieces of leaves were post fixed in 1% OsO_4 at 0–4°C for 2 h. The material was dehydrated in ethanol, than propylene oxide and afterwards embedded in Spurr–Epon mixture. Ultrathin sections cut on a Reichert Ultratome were stained with uranyl acetate and lead citrate according to Reinolds (1963) procedure and then examined by Jeol 100 CX electron microscope, Japan. Three digital TEM micrographs were taken from random cells, these magnification were chosen to discern clearly the structure of the chloroplasts.

HPLC analysis of α- tocopherol, ascorbic acid and ß-carotene

Extraction

Alpha-tocopherol was extracted according to the method of Ubaldi *et al*. (2005). *Azolla* fronds were extracted with 30 ml ether (twice). Ether phases were combined and transferred in the separating funnel, rinsed 6 times with 50 ml water and recovered in a round-bottom flask. Separating funnel was rinsed with 10 ml ether recovered in a round-bottom flask. Then, the test material was evaporated to dryness in a rotary evaporator under partial vacuum at water-bath at 45 ^oC for 5 min. After cooling, test material was recovered with 5 ml methanol, well mixed and transferred in a glass tube, centrifuged at 4000 rpm for 5 min.

Ascorbic acid (vitamin C) was extracted according to the modified method of Abdulnabi *et al*. (1997). Ten gram of *Azolla* fronds were homogenized with an extracting solution containing meta-phosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask (wrapped with aluminum foil) and agitated at 100 rpm with the aid of an orbital shaker for 15 min at room temperature. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract.

The ß-carotene in the sample was extracted according to the method described by Tee et al. (1996) with slight modification (Ismail and Fun, 2003). The sample (10 g) was added with 40 ml of 99.8% ethanol and 10 ml of 100% (w/v) potassium hydroxide, and homogenized for 3 min using a blender. The mixture was saponified by means of a refluxing apparatus, and heated using a heating mantle for 30 min, and then cooled to room temperature. The mixture was frequently agitated to avoid any aggregation. For the extraction step, the mixture was transferred into a separation funnel and 50 ml of n-hexane was added. The funnel was inverted, vented and then shaken vigorously for a few seconds, and the layers were allowed to separate. The upper layer (hexane extract) was pipettered out, and the aqueous layer was re-

extracted twice, each time with 50 ml of n-hexane. The extract was then filtered through anhydrous sodium sulphate to remove any water residue.

Analysis

Alpha-tocopherol, vitamin C and ß-carotene were determined by a reverse-phase HPLC technique. Hewlett Packard HPLC Series 1100 (USA) equipped with degasser, quaternary pump, auto-sampler and diode array detector was used. A Ultrasphere octadecylsilyl (ODS) Hypersil C_{18}, 5 mm particle size, in a 250 mm length x 4.0 mm I.D stainless steel column (Hewlett Packard) was used. The separation and identification conditions for the antioxidants are tabulated in Table 1.

Table 1: HPLC conditions for separation and identification of vitamin E, vitamin C and beta-carotene.

Parameters	Conditions		
	Vitamin E	Vitamin C	ß-carotene
Mobile phases	-Methanol	-0.1 M potassium acetate, pH 4.9 -Acetonitrile-water (50:50)	- Acetonitrile- Methanol-ethyl acetate (88-10-2)
Flow rate	1 ml/min	1.5 ml/min	1 ml/min
Detection	294 nm	254 nm	250 nm

RESULTS AND DISCUSSION

The maximal daily UV-B doses varied between 1 and 2 KJ $m^{-2}d^{-1}$, calculated by Behrenfeld *et al*. (1993) from incident irradiance at the Pacific Ocean's water surface in summer at med-latitudes. In our experiment the UV-B dose was reached to nearly 2.5 folds increase more than the maximal daily doses.

The most striking effect of the enhanced UV-B dose treatment on the ultrastructure of *A. caroliniana* was observed after 48 h exposure period as markedly widened chloroplast area occupied by large amount of plastoglobuli in stressed fronds (Fig. 1). On the other hand, plastoglobules were rarely or occasionally detected in the chloroplasts of the control fronds. The chloroplast membrane got significantly altered across treatment, especially those exposed to UV-B for 48 h relative to the control.

Deformation of chloroplasts structures may be due to oxidative damage under UV-B radiation. Stoyanova and Velikova (1997) and Tarhanen (1998) suggested that ROS disturb the ultrastructure of cell organelles, mostly plastids and mitochondria. In addition, the changes in chloroplast size followed by marked changes in the area of starch; also one notable difference in this study is that starch was readily detected in controlled grown *Azolla caroliniana*, while they were rarely observed in the UV-B treated *Azolla* (Fig.1). Lack of starch in the chloroplasts in response to UV-B exposure suggests little photosynthetic activity of the fronds towards the UV-B exposure. Plastoglobuli (lipoprotein sub-compartments structure) consist mainly of triacylglycerols, plastohydroquinone and α-tocopherol and it has been reported that their numbers increase during the up-regulation of plastid lipid metabolism in response to different stresses and during senescence (Murphy, 2001). In our experiment plastoglobules size, number and intensities of *A. caroliniana* subjected to UV-B radiation were linearly increased with the UV-B dose till the end of the experiment. These finding are in agreement with observations of the increase in the number and/or size of plastoglobules in response to abiotic stresses in grapevine (Britvec *et al*., 2001). Also, the cell wall increased in thickness under elevated UV-B exposure for 24 h, then completely deformed after 48 h (Ibrahim and Mostafa, 2001). Thinner cell walls under UV-B exposure may result from a delay in cell wall differentiation (Fig.1). Cell wall measurements are also prone to inaccuracies because

the cell wall thickness varies considerably between and within cells. Similar observations in needles of *Norway spruce* were discussed by Kivimaenpaa *et al.* (2003). Ibrahim and Mostafa (2007) explained that after application of UV-B radiation for 48 h on *Azolla* plant, the content of deformed chloroplasts increased up to 95% and the cell cross-sectional area occupied by chloroplasts was significantly increased in a linear manner with increasing the duration of exposure to UV-B.

Chromatographic characterization of α-tocopherol showed great quantative variations, whereas, ascorbic acid and ß-carotene showed marked changes in both number and area of the characterized peaks. In our study, the chromatogram of α-tocopherol showed three different peaks throughout the UV-B exposure periods (Fig. 2). The peaks area were reduced after exposure of *Azolla* fronds to UV-B radiation for 48 h reached to 60% and 41% for peak 1 and 3, respectively in comparison to its exposure for 24 h (Table 2). On the other hand, there were an increase in peaks 1 and 3 area reached to 33% and 13% after exposure of *Azolla* fronds to UV-B radiation for 24 h in comparison to control, respectively.

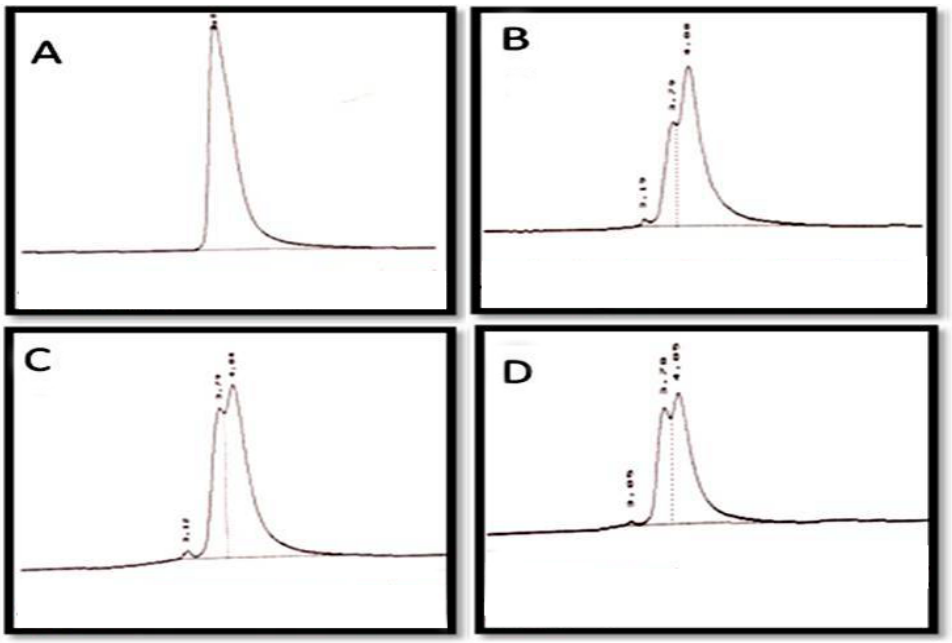

Fig. 2: Chromatogram of an extract of *Azolla caroliniana* obtained by HPLC. The α-tocopherol was readily detected and quantified in this representative sample collected after the exposure of fronds to UV-B radiation for 24 and 48 h. Assignment of peaks was conducted by comparing the retention time obtained for standards with the analysis of the characteristic peaks from the samples. A, standard; B, control; C and D, exposure to UV-B for 1 and 2 d, respectively.

These results suggested the involvement of α-tocopherols in the photoprotection of *A. caroliniana* against the oxidative stress caused by UV-B radiation.

Our results are consistent with the findings of other authors who found that the level of α-tocopherol increases in plants that exposed to environmental stress which are susceptible to induce oxidative stress (Wildi and Lütz, 1996; Delong and Steffen, 1997; Fryer *et al*., 1998; Havaux *et al*., 2000 and Munné-Bosch and Alegre, 2000). Ascorbic acid is the most important metabolite, universal in photosynthetic eukaryotes (Smirnoff, 1996). After exposure of *A. caroliniana* fronds to UV-B radiation for 48 h, peaks area for ascorbic acid were all markedly affected and reduced to a values reached 34, 33 and 37.5 %, respectively in comparison to its exposure for 24 h (Table 2).

Table 2: Chromatographic profile of α-tocopherol, ascorbic acid and β-carotene in *Azolla caroliniana* subjected to UV-B radiation for 24 and 48 h. n.d., not detected.

Compound	Time	Retention Time [min]			Peak area [Cm^2]		
		Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
α-tocopherol	Control	3.23	3.78	4.03	15×10^{-4}	0.052	0.15
	24 h	3.19	3.79	4.08	20×10^{-4}	0.054	0.17
	48 h	3.05	3.78	4.05	8×10^{-4}	0.056	0.10
Ascorbic acid	Control	4.34	5.22	n.d.	0.050	0.015	n.d.
	24 h	4.56	5.46	5.89	0.046	0.012	0.008
	48 h	4.60	5.47	5.89	0.030	0.008	0.005
β -Carotene	Control	3.18	3.76	------	7×10^{-4}	0.16	------
	24 h	3.19	3.76	------	8×10^{-4}	0.15	------
	48 h	n.d.	3.75	------	n.d.	0.13	------

In control plants, two peaks only were detected (Fig. 3). Selvakumar (Selvakumar, 2008) recorded that UV-B treatment increased proportionally the ascorbic acid content of *Vigna unguiculata* and *Crotalaria juncea* plants depending on the dose used.

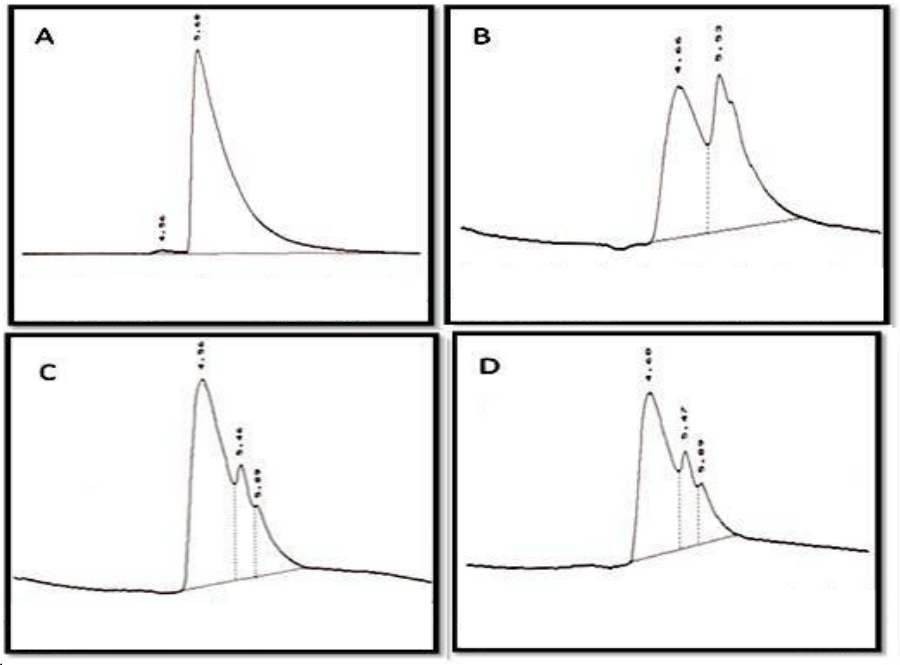

Fig. 3: Chromatogram of an extract of *Azolla caroliniana* obtained by HPLC. The ascorbic acid was readily detected and quantified in this representative sample collected after the exposure of fronds to UV-B radiation for 24 and 48 h. Assignment of peaks was conducted by comparing the retention time obtained for standards with the analysis of the characteristic peaks from the samples. A, standard; B, control; C and D, exposure to UV-B for 1 and 2 d, respectively.

Also, Kumari *et al*. (2010) observed an increase in ascorbic acid content of *Acorus calamus*, a medicinal plant, due to UV-B exposure at initial stage of sampling,

whereas at later stages a decline was observed. Ascorbic acid acts as antioxidant, reacting directly with hydroxyl radical, single oxygen and superoxide radicals. Increase in ascorbic acid in plants after UV-B exposure compared with untreated plants were also manifested in several studies suggesting its induction due to UV-B stress (Hernandez *et al*., 1995; Costa *et al*., 2002 and Nasibi and Kalantari, 2005). The reduction in ascorbic acid could be explained due to increase activity of ascorbate peroxidase after UV-B exposure resulting into more consumption of ascorbic acid for effective quenching of oxyradicals (Kumari *et al*., 2010). Decline in ascorbic acid under UV-B stress was also reported by Agrawal and Rathore (2007) in wheat and mung bean.

After 24 h exposure of *Azolla* fronds to UV-B radiation, two peaks of ß-carotene were characterized at retention time 3.19 and 3.76 min (Table 2). Whereas, the prolonged exposure to UV-B for 48 h resulted in a complete disappearance of the first peak and also a reduction in area of the second peak, compared with control plants and that exposed for 24 h to UV-B radiation (Fig. 4). Similarly, Hernando *et al*. (2005) suggested that exposure of *Asteromonas sp.* to UV-B and UV-A radiation for 3 d resulted in an increase in ß-carotene content and a marked decrease was observed afterwards. ß-carotene can directly quench singlet oxygen or prevent the formation of a chlorophyll triplet excited state (Young et al., 1997). The changes in *A. caroliniana* fronds after its exposure to UV-B radiation for 48 h could indicate severe effects which resulted in a decrease in ß-carotene synthesis, suggesting its role as antioxidant in protection against injuries.

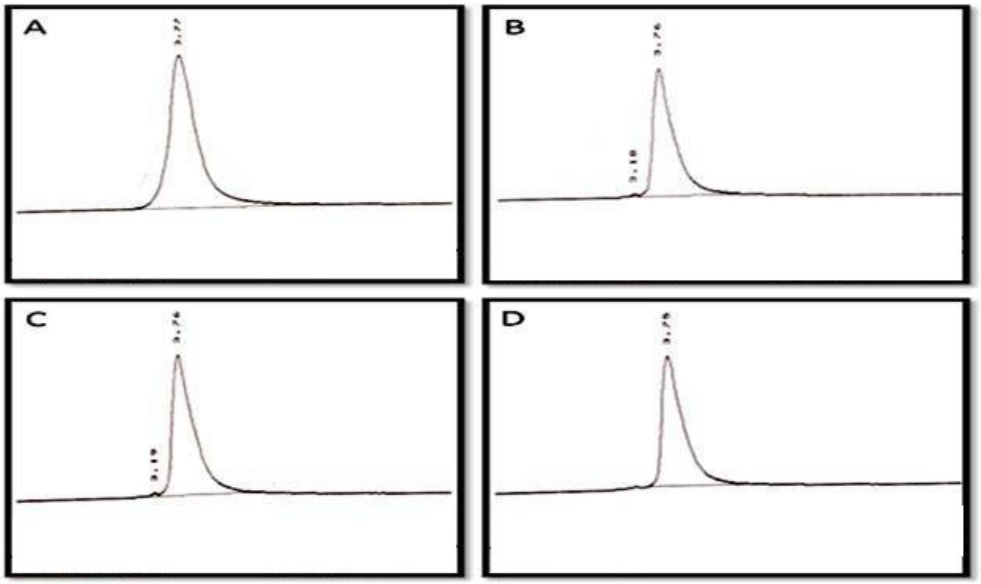

Fig. 4: Chromatogram of an extract of *Azolla caroliniana* obtained by HPLC. The β-carotene was readily detected and quantified in this representative sample collected after the exposure of the fronds to UV-B radiation for 24 and 48 h. Assignment of peaks was conducted by comparing the retention time obtained for standards with the analysis of the characteristic peaks from the samples. A, standard; B, control; C and D, exposure to UV-B for 1 and 2 d, respectively.

This study reports ultrastructural responses of *Azolla caroliniana* to prolonged exposure to UV-B radiation. The observed responses throughout the experiment indicated that prolonged exposure to UV-B radiation resulted in a marked deformation in the ultrastructure of chloroplast, which may be resulted in the destruction of photosynthetic activity. This damage indicated that activation of various antioxidants

did not provide complete protection to *Azolla caroliniana*, especially which exposed to UV-B radiation for 48 h.

REFERENCES

Abdulnabi, A.A.; Emhemed, A.H.; Hussein, G.D. and Biacs, P. A. (1997). Determination of antioxidant vitamins in tomatoes. Food Chem. 60: 207-212.

Agrawal, S.B. and Rathore, D. (2007). Changes in oxidative stress defense system in wheat (Triticum aestivum L.) and mung bean (Vigna radiate L.) cultivars grown with and without nutrient and irradiated by supplemental ultraviolet-B. Environ. Exp. Bot. 59: 21-33.

Asada, K. (1994). The water-water cycle in the chloroplasts: Scavenging of active oxygen and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant mol. Biol. 50: 601-639.

Behrenfeld, M.; Hardy, J.; Gucinski, H.; Hanneman, A.; Lee, H. and Wones, A. (1993). Effects of ultraviolet-B radiation on primary production along latitudinal transects in the South Pacific Ocean. h4ar Environ Res. 35: 349-63.

Britvec, M.; Reichenauer, T.; Soja, G.; Ljubesic, N.; Eid, M. and Pecina, M. (2001). Ultrastructure changes in grapevine chloroplasts caused by increased tropospheric ozone concentrations. Biologia Bratislava. 56:417-424.

Burton, G.W.; Joyce, A. and Ingold, K.U. (1982). First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma. Lancet. 2:327.

Costa, H.; Gallego, S. M. and Tomaro, M. L. (2002). Effects of ultraviolet-B radiation on antioxidant defense system in sunflower cotyledons. Plant Sci. 162: 939-945.

Delong, J.M. and Steffen, K.L. (1997). Photosynthetic function, lipid peroxidation and α-tocopherol content in spinach leaves during exposure to UV-B radiation. Can J Plant Sci. 77: 453-459.

El-Aggan, W.H. (1982). A comparative study of the growth and nitrogenase activity of five *Azolla* species as affected by various environmental factors. Ph.D. Thesis, Universite Catholique De Louvain.

Foyer, C.H.; Lelandais, M. and Kunert, K.J. (1994). Photooxidative stress in plants. Physiol. Plant. 92: 696–717.

Fryer, M.J.; Andrews, J.R.; Oxborough, K.; Blowers, D.A. and Baker, N.R. (1998). Relationship between CO_2 assimilation, photosynthetic electron transport, and active O-2 metabolism in leaves of maize in the field during periods of low temperature. Plant Physiol. 116: 571-580.

Havaux, M.; Bonfils, J.P.; Lütz, C. and Niyogi, K.K. (2000). Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the *npq1* Arabidopsis mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase. Plant Physiol. 124: 273-284.

Hernandez, J. A.; Olmos, E.; Corpas, F. J.; Sevilla F. and del Rio L. A. (1995). Salt-induced oxidative stress in chloroplast pea plants. Plant Sci. 105: 151-167.

Hernando, M.P.; Malanga, G.F. and Ferreyra, G.A. (2005); Oxidative stress and antioxidant defenses generated by solar UV in a Subantarctic marine phytoflagellate. Sci. Mar. 69: 287-295.

Holopainen, T.; Anttonen, S.; Palomaki V.; Kainulainen, P. and Holopainen, J. K. (1996). Needle ultrastructure and starch content in Scots pine and Norway spruce after ozone fumigation. Can. J. Bot. 74: 67-76.

Holopainen, T.; Anttonen, S.; Wulff, A.; Palomaki V. and Karenlampi L. (1992). Comparative evaluation of the effects of gaseous pollutants, acidic deposition

and mineral deficiencies: structural changes in the cells of forest plants. Agric. Eco. Eviron. 42: 365-398.

Ibrahim, M. M. and Mostafa, E. M. (2007). UV-B effect on constituents of *Azolla caroliniana*. Z. Naturforsch. 62 (c): 246-252.

Ibrahim, M. M. and Bafeel, S. O. (2011). Molecular and Physiological Aspects for *Lepidium sativum* Tolerance in Response to Lead Toxicity. Fresenius Environmental bulletin. 20 (8): 1871-1879.

Ismail, A. and Fun, C.S. (2003). Determination of vitamin C, ß-carotene and Riboflavin contents in five green vegetables organically and conventionally grown. Mal J Nutr. 9(1): 31-39.

Kakani, V.G.; Reddy, K.R.; Zhao, D. and Sailaja (2003); Field crop response to ultraviolet –B radiation: A review. Agric. For. Meteorol. 120: 191-218.

Kivimaenpaa, M.; Sutinen, S.; Karlsson, P.E. and Sellden G. (2003). Cell structural changes in the needles of Norway spruce exposed to long term ozone and drought. Annals of Botany. 92: 779-793.

Kumari, R.; Singh, S. and Agrawal, S. B. (2010). Response of ultraviolet-B induced antioxidant defense system in a medical plant, *Acorus calamus*. J. Environmental Biology. 31: 907-911.

Lesser, M. P.; Cullen, J. J. and Neale, P. J. (1994). Photoinhibition of photosynthesis in the marine diatom *Thalassiossira pseudonono* during acute exposure to ultraviolet–B radiation: relative importance of damage and repair. J. Phycol. 30: 183-192.

Lowe, G.M.; Booth, L.A.; Young, A. J. and Bilton, R. F. (1999). Lycopene and beta-carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses. Free Radic Res. 30 (2): 141-151.

Lumpkin, T.A. and Plucknett, D.L. (1982). *Azolla* as a Green Manure: Use and Management in Crop Production. Westview Tropical Agriculture Series, Westview, Boulder, 230 pp.

Munné-Bosch, S. and Alegre, L. (2000). Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta. 210: 925-931.

Murphy, D. J. (2001). The biogenesis and functions of lipid bodies in animals, plants and microorganisms.- Progress in Lipid Research. 40: 325-438.

Nasibi, F. and Kalantari, K.M. (2005). The effects of UV-A, UV-B and UV-C on protein and ascorbate content, lipid peroxidation and biosynthesis of screening compounds in *Brassica napus.* Iran. J. Sci. Technol. Transaction A-Science Winter. 29: 39-48.

Reinolds, S.S. (1963). The use of lead citrate of high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208–212.

Sanginga, N. and Van Hove, C. (1989). Amino acid composition of *Azolla* as affected by strains and population density. Plant and Soil. 117: 263-267.

Selvakumar, V. (2008). Ultraviolet-B radiation (280-315 nm) invoked antioxidant defense systems in *Vigna unguiculata* (L.) Walp. And *Crotalaria juncea* L. Photosynthetica. 46: 98-106.

Singh, N.; Ma, L.Q.; Srivastava, M. and Rathinasabapathi, B. (2006). Metabolic adaptations to arsenic induced oxidative stress in *Pteris vittata* and *Pteris ensiformis* L. Plant Sci. 170: 274-282.

Smirnoff, N. (1996). The function and metabolism of ascorbic acid in plants. Ann. Bot. 78: 661-667.

Solomon, K.R. (2008). Effects of ozone depletion and UV-B radiation on humans and the environment. Atmos. Ocean. 46: 185-202.

Stoyanova, D. and Velikova, V. (1997). Effects of simulated acid rain on chloroplast ultrastructure of primary leaves of *Phaseolus vulgaris*. Biol. Plant. 40:589–595.

Tarhanen, S. (1998). Ultrastructural responses of the lichen *Bryoria fuscesens* to simulated acid rain and heavy metal deposition. Ann. Bot. 82:735–746.

Tee, E.S.; Kuladevan, R.; Young, S.I.; Khor, S.C. and Zakiyah, H.O. (1996). Nutrient analysis of foods. Kuala lumpur: Institute Medical for Research.

Ubaldi, A.; Delbono, G.; Fusari, A. and Serventi, P. (2005). Quick HPLC method to determine vitamin E concentration in cow's milk. Ann. Fac. Medic. Vet.di Parma. 25: 101-110.

Van Hove, C.; de Waha Baillonville, T.; Diara, H.F.; Godard, P.; Mai Kodomi, Y. and Sanginga, N. (1987). *Azolla* collection and selection. *Azolla* Utilization. In: Proceedings of the Workshop on *Azolla* Use, Fuzhou, Fujian, China, 31 March – 5 April, 1985. Int. Rice Res. Inst., Los Banos, Philippines, pp. 77-87.

Wang, Y.; Feng, H. Qu, Y.; Cheng, J.; Zhao, Z.; Zhang, M.; Wang, X. and An, L. (2006). The relationship between reactive oxygen species and nitric oxide in ultraviolet–B–induced ethylene production in leaves of maize seedlings. Environ. Exp. Bot. 57: 51-56.

Wildi, B. and Lütz, C. (1996). Antioxidant composition of selected high alpine plant species from different altitudes. Plant Cell Environ. 19:138-146.

Young, A.J.; Phillip, D. and Savill, J. (1997). Carotenoids in higher plant photosynthesis. In: Pessaraki M. (ed.), Handbook of photosynthesis, pp. 575- 96. Marcel Dekker, New York.

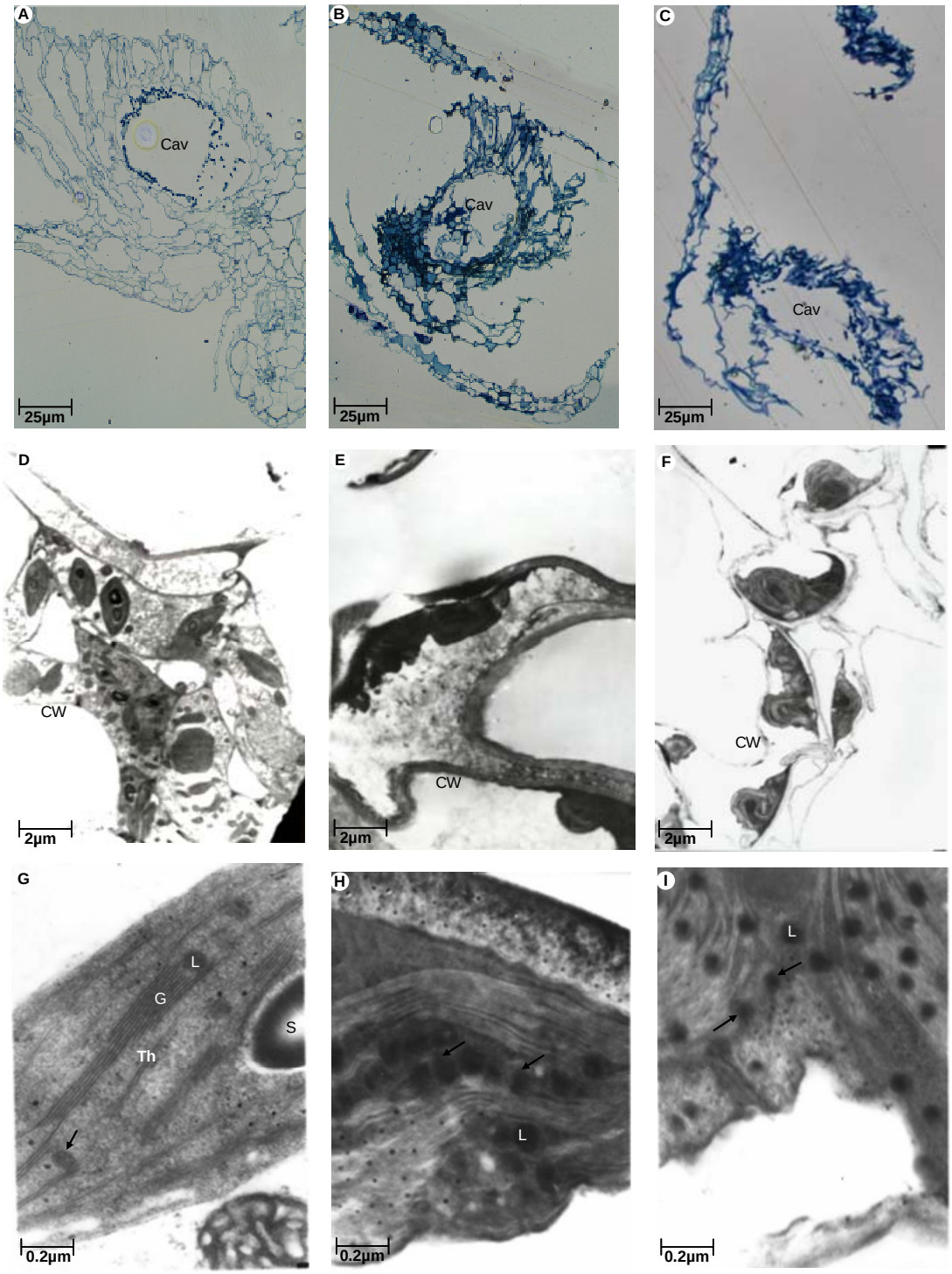

Fig.1: Microscopic photographs (A-C) and electron micrographs (D-I) from *A. caroliniana* (A) control plants. (B) Cells after 24 h UV-B treatment. (C) Cells after 48 h UV-B treatment. (D, G) Control plants, detail of chloroplasts showing large starch granules and few plastoglobuli. (E, H) Cells after 24 h UV-B treatment, detail of chloroplasts showing few starch granules and large amount of plastoglobuli. (F, I) Cells after 48 h UV-B treatment detail of chloroplasts showing swollen chloroplasts with low density of stroma and strongly wave like thylakoid and most abundant of plastoglobuli. Arrows point to plastoglobuli; Cav, cavity including *Anabaena*; CW, Cell wall; G, granum stack; L, lipid bodies; S, starch granule; Th, thylakoid.

ARABIC SUMMARY

التحليل الكروماتوجرافي للمركبات المضادة للأكسدة في نبات الأزولا المعرض للأشعة فوق البنفسجية

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نبات الأزولا تم تعريضه لجرعات مختلفة من الأشعة فوق البنفسجية ولفترات محددة وقد وجد أن التركيبات فوق الخلوية للبلاستيدات الخضراء في النبات قد تأثرت بشكل معنوي واضح. كذلك المركبات المضادة للأكسدة التي تم تحديدها مثل مركب الألفاتوكوفيرول، حمض الأسكوربيك والبيتا كاروتين، تم فصلها وتعريفها باستخدام تقنية الفصل الكروماتوجرافي السائل تحت الضغط العالي. وبمقارنة المنحنيات الخاصة والتي تم فصلها لهذه المركبات بعد تعرض نبات الأزولا لجرعات وأوقات مختلفة وجد اختلافات وتباينات معنوية في هذه المركبات نتيجة لإختلاف الأوقات والجرعات التي تعرض لها النبات.النتائج أظهرت أن تعرض نبات الأزولا لجرعات من الأشعة الفوق بنفسجية لمدة 48 ساعة أدت إلى تغيرات معنوية واضحة في البلاستيدات الخضراء من خلال زيادة مساحتها، بالإضافة إلى زيادة أعداد البلاستوجلوبيل في النباتات المعرضة لجرعات عالية من الأشعة. زيادة الجرعات التي تعرض لها نبات الأزولا لمدة 48 ساعة أدت إلى نقص في مساحة المنحنى الخاص بالمركبات المضادة للأكسدة سالفة الذكر. من خلال هذه النتائج المتحصل عليها نستنتج أن تغير كميات وأنواع المركبات المضادة للأكسدة في نبات الأزولا تعتمد على الجرعة والوقت الذي يتعرض له النبات للأشعة فوق البنفسجية. وهذه المركبات لها دور كبير في حماية نبات الأزولا لحد معين مقاوماً للأشعة فوق البنفسجية.