Applied Biological Research 14 (2): 202-206, 2012 **Short communication**

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE OF *Azolla filiculoides* TO THE EXPOSURE OF ULTRA VIOLET-β RADIATIONS

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ABSTRACT

The effect of UV-β radiations on some growth and biochemical parameters of aquatic fern *Azolla filiculoides* was assessed for induced instability in the fern. UV-β radiation @ 6.8 Wm^{-2} was made incident on the fronds of fern for various periods (20, 40 and 60 min). Plants exhibited mild to severe injury symptoms and the extent of injury was estimated by observing the visible symptoms of chlorosis and necrosis. Fern biomass, doubling time, average root number and average root length was also measured. Severe chlorotic injury followed by necrosis occurred in the plants exposed to UV-β radiations for 60 min. With increase in exposure time to UV-β radiation the fern biomass, average root number and average root length were significantly reduced with associated increase in doubling time. 5-amino laevulinic acid (the precursor of chlorophyll) content of *A. filiculoides* was severely reduced in all exposure periods resulting in less chlorophyll biosynthesis by plant. The carotenoid content increased at no or mild injury but decreased due to severe injury. Starch, sugar and protein contents showed decreasing trend with increase in exposure time. Preliminary observations indicated that the disturbances in photosynthetic machinery lead to decrease in biomass production revealing physiological stress on *A. filiculoides* due to UV-β radiations.

Keywords: *Azolla filiculoides,* biomass, chlorophyll, chlorosis, necrosis, UV-β radiation

INTRODUCTION

Azolla, a small aquatic pteridophyte, is used as biofertilizer in rice fields as it fixes nitrogen due to the presence of cyanobacteria in the dorsal leaf cavity of host. *Azolla-anabaena* symbiosis has proved most promising and efficient system in sustaining production in low input cultivation or where little or no combined nitrogen is available especially in paddy fields. In view of increased chemical pollution threat to environment due to chemical fertilization, *Azolla-anabaena* system could be successfully exploited to boost agricultural productivity. In recent years emphasis has been laid on the use of *Azolla* in paddy fields (Stefano and Antonino, 2010) but little attention has been paid to the response of *Azolla* to the exposure of UV-β radiations. The general erosion of stratospheric O_3 layer due to the increased industrialization and urbanization has been responsible for increase in UV-β radiation on earth's surface which adversly affects the ecosystem (Bjorn *et al*., 1999; Sheo and Sushil, 2011). Approximately 90% of incoming UV-β radiation is absorbed by ozone layer, but this absorption varies significantly with ozone concentration. A relatively small decrease in ozone concentration corresponds to a large increase in UV-β transmittance (Thomas and Patrick, 2002).

UV-β radiation can have wide-ranging negative impacts on living organisms including plants (Frohnmeyer and Stager, 2003; Susan *et al*., 2003). The physiological and biochemical effects of UV-β (280 - 315 nm) radiation on higher and lower plants have been reviewed by various workers (Thomas and Patrick, 2002). In cyanobacteria and algae, UV-β radiations affect growth particularly the physiological processes such as photosynthesis, etc. (Jayakumar *et al*., 2002). Growth characteristics such as shoot length, root length or leaf area are altered in higher plants exposed to UV-β radiations. These radiations may damage DNA, proteins and membranes, alter transpiration and photosynthesis and induce changes in growth, development and morphology (Whelan and Glaser, 1997). In spite of the attempts to deplete ozone layer, the level of UV-β radiations in environment is on rise (Jayakumar *et al*., 2002). Very little information is available on the response of *Azolla* plants to UV-β radiations. Keeping in view the importance of *Azolla*, an attempt was made to evaluate the disturbances caused in *Azolla* by enhanced UV-β radiation.

MATERIALS AND METHODS

Azolla filiculoides was procured from the National Center for Culture Collection and Utilization of Blue Green Algae, IARI, New Delhi, India and maintained in tubs containing combined nitrogen-free 2/5 strength sterile Hoagland's medium with micro-nutrients (Allen, 1968). The pH was adjusted to 5-6 by using 0.04 mM FeEDTA. Culture was grown at 28±1°C under 16:8 (light: dark) photoperiod using cool white fluorescent lamps at a photon fluence rate of 95 μmol m^{-2} twice a week. The cultures in log phase were transferred to sterilized petri-dishes and irradiated at 6.8 Wm^{-2} for 20, 40 and 60 min. in dark chamber using UV-β (320 nm) emitting florescent tubes (Philips TL 20 W/12) placed at a distance of 30 cm above the petri-dishes. The plants were observed for 72 h for visible injury symptoms, if any. Accordingly, the plants were categorized into no injury, mild injury and severe injury for comparison. Fifteen plants from each petri-dish were analyzed after 72 h exposure. Plants unexposed to UV-β radiation served as control. Biomass of *Azolla* fronds was determined in terms of dry weight and doubling time calculated by using the formula given by Subudhi and Watanabe (1981).

$$\text{Doubling time (d)} = t\, r^{-1}$$

where, t = experimental period, r = log (M_1/M_0)/0.301, M_1 = mass of culture after treatment, M_0 = mass of the initial culture, and 0.301= constant.

For pigment analysis, *A. filiculoides*, blotted dry on filter papers, was ground in 80% acetone and allowed to stand in dark at 4°C overnight for complete extraction and centrifuged at 8000 rpm for 5 min. The amount of chlorophyll in the supernatant was determined as per the method of Lichtenthaler (1987). The caroteniod content was determined as per Duxbury and Yentshc (1956). For determination of 5-amino-laevulinic acid (ALA), the method of Stobart *et al*. (1985) was adopted. The concentration of ALA was determined using the calculated extension coefficient $7.24 \cdot 10^4$. For protein estimation, plants were homogenized in chilled phosphate buffer (0.1 M, pH 7.0) using pestle and mortar, muslin filtrated and the homogenate centrifuged at 6000 rpm for 5 min. The supernatant was used to determine the protein content (Lowry *et al*., 1951). Total free sugar was estimated chlorimetrically in alcoholic extract (Montogomery, 1957) and the residue left after alcoholic extraction was hydrolyzed with perchloric acid to estimate starch content (Agarwal *et al*., 1977)**.** The data was analyzed by using ANOVA and the differences between treatment means analyzed at 5% level of significance.

RESULTS AND DISCUSSION

UV-β radiation exposure lead to an array of physiological changes in the organism and the severity of symptoms depended upon the exposure time. UV-β radiation exposure induced visible injury symptoms in *A. filiculoides* in the form of chlorosis, necrosis or both (Table 1 and 2). Plants exposed to UV-β

radiation for 20 min. did not show any injury sign. However, the plants exposed to UV-β radiation for 40 min. showed visible symptoms of chlororsis and marginal necrosis (mild injury). Injury symptoms were severe in plants exposed to UV-β radiation for 60 min. Young fronds were highly susceptible to injury than the older ones. The higher sensitivity of young fronds may be due to the involvement of phenols and lower carotenoid content to ward off UV-β radiation (Howel, 1974; Farooq *et al*., 2000).

Table 1: Effect of UV-β radiation on biomass, doubling time, average root number and average root length of *Azolla filiculoides*

Exposure time (min.)	Dry biomass weight (g)	Doubling time (d)	Average root number frond^{-1}	Average root length (mm)
0	0.9	3.0	0.9	4.2
20	0.7	3.2	0.7	3.7
40	0.5	3.5	0.5	3.4
60	0.3	4.0	0.3	3.0
$CD_{0.05}$	0.3	0.1	0.4	0.9

Azolla filiculoides exposed to UV-β radiation showed significant changes in growth parameters studied such as biomass, doubling time, average root number and average root length. Algal biomass was estimated as the increment in dry weight. Biomass decreased by 22.2, 44.4 and 66.6% in plants exposed to UV-β radiations for 20, 40 and 60 min., respectively (Table 1). Reduction in growth was evident in terms of increase in doubling time and decrease in average root number and root length. The doubling time in unexposed plants was 3.0 d but increased to 3.2, 3.5 and 4.0 d in the plants exposed to UV-β radiation for 20, 40 and 60 min., respectively; reflecting 6.6, 16.6 and 33.3% increase in doubling time. Jayakumar *et al*. (2002) also observed reduction in biomass and increase in doubling time in *Azolla microphylla* plants exposed to UV-β radiation. Increase in doubling time indicated the reduced growth and lag in the accumulation of biomass. The reduction in biomass and growth could be correlated to the inhibition in photosynthesis. The low photosynthetic pigment of *A. filiculoides* (Table 2) under UV-β exposure suggests possible reduction in photosynthesis. The enhanced UV-β radiation inhibited photosynthetic activity through direct absorption by leaves. Low photosynthetic activity in A*zolla* plants may also be attributed to the reduced activity of Rubisco, the key enzyme involved in CO_2 fixation (Jun and Tadashi, 2006).

A gradual decrease in total chlorophyll content (chl*a* + chl*b*) was observed in UV-β treated *Azolla* plants (Table 2). As compared to the unexposed *Azolla* plants, the decrease in total chlorophyll content was 16.3, 38.1 and 48.0% in UV-β irradiated plants for 20, 40 and 60 min., respectively. The reduction in total chlorophyll content may be correlated with the reduction in its precursor ALA as evident from the experiment. ALA content showed a decreasing trend with increase in exposure time of *Azolla* plants to UV-β radiation. The carotenoid content, however, was not affected at low dose (20 min. exposure) of UV-β; rather it showed significant increase as compared to unexposed control. Beyond 20 min. exposure, the carotenoid content decreased at 40 and 60 min. UV-β irradiation, with maximum reduction at 60 min. of exposure depicting 20.2% decrease over control. Enhancement of caroteniod content at 20

Table 2: Effect of UV-β radiations on chl*a*, chl*b*, chl*a* + chl*b*, chl*a*/hl*b*, ALA and carotenoid contents of *Azolla filiculoides*

Exposure time (min.)	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Chl *a* + Chl *b* (mg g^{-1} FW)	Chl *a*/ Chl *b*	ALA content (µ mol g^{-1} FW)	Carotenoid content (mg g^{-1} FW)
0	0.421	0.203	0.624	2.073	151.1	0.198
20	0.355	0. 167	0.522	2.125	149.2	0.226
40	0.212	0.174	0.386	1.218	142.2	0.178
60	0.201	0.123	0.324	1.634	135.4	0.158
$CD_{0.05}$	0.201	0.125	0.243	0.932	0.612	0.015

min. UV-β exposure could be attributed to the protection of photosynthetic apparatus (Dohler, 1998), but decrease in caroteniod content at 40 and 60 min. exposure indicates the failure to protect the photosynthetic apparatus at enhanced exposure. It has been proposed that photosynthetic pigments are bleached by UV-β irradiation and converted to pheophytin (Hauder *et al*., 1991).

Table 3: Effect of UV-β radiations on starch, sugar and protein contents of *Azolla filiculoides*

Exposure time (min)	Starch content (mg g^{-1} FW)	Sugar content (mg g^{-1} FW)	Protein content (mg g^{-1} FW)
0	76.2	0.62	41.0
20	70.1	0.30	27.2
40	66.1	0.28	23.5
60	60.2	0.25	15.3
$CD_{0.05}$	0.9	0.02	0.40

A marked decrease in the metabolites such as starch, sugar and protein contents of *Azolla* was observed due to UV-β exposures for different time (Table 3). Maximum reduction was noted at 60 min. exposure of *Azolla* plants to UV-β radiations. Starch and sugar contents were determined to have idea about the rate of photosynthesis of *Azolla* under UV-β irradiation. The reduced sugar accumulation in *Azolla* plants may be due to the limited rate of photosynthesis. Similarly, the protein content in *Azolla* exposed to UV-β radiations was adversely affected as compared to the unstressed plants. Maximum reduction (62.6%) in protein content was noted at 60 min. exposure. Proteins are highly sensitive to UV-β due to their peak absorbance range in UV region (Caldwell, 1979). Decrease in protein content in plants due to UV-β exposure is a general response (Hauder and Figueroa, 1997; Dohler *et al*., 1998; Suresh Babu *et al*., 1998). The increased hydrolysis of protein and starch has been proposed as the main reason for depletion in protein and starch contents under UV-β exposure and this may be an adaptation of *Azolla* plants to meet its energy requirement for various physiological and biochemical processes when photosynthesis is not going on at its full potential as has been found in this study.

Conclusion: The study revealed that it is the disturbance in the rate of photosynthesis that leads to the reduced growth of *Azolla* plants when exposed to UV-β radiation. In addition, the reduction in carotenoid content renders *Azolla* plants unable to protect themselves from the oxidative stress induced by higher exposures of the plant to UV-β radiation.

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