Effects of enhanced ultraviolet-B (280-320 nm) radiation on growth and photosynthetic activities in aquatic fern *Azolla microphylla* Kaulf.

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Abstract

The effects of enhanced UV-B radiation on growth and photosynthetic activities were investigated in fronds of the aquatic fern *Azolla microphylla* Kaulf. The fronds were exposed to UV-B radiation intermittently once in 3 d during 12 d. Biomass and relative growth rate of UV-B treated *Azolla* plants and the heterocyst frequency of the UV-B treated symbiont decreased resulting in an increase in doubling time over the control. The doubling time was 3.08 d for control and 3.35 d for UV-B irradiated plants. Chl and carotenoid contents per unit fresh mass and photosystem 2 (PS2) activity also decreased under UV-B treatment. Measurements of photosynthetic activity in terms of fluorescence kinetics and PS2 mediated O₂ evolution showed that the aquatic fern *Azolla* is sensitive to UV-B damage.

Additional key words: biomass; carotenoids; chlorophyll; doubling time; fluorescence induction; heterocyst; photosystem 2; relative growth rate.

Introduction

The physiological and biochemical effects of UV-B (280-320 nm) radiation on higher and lower plants were reviewed by Klein (1978) and Häder and Figueras (1997). In cyanobacteria and algae, UV-B radiation affects growth and several other physiological processes including photosynthesis (Döhler and Alt 1989, Wilhelm et al. 1997). Growth characteristics such as shoot height, root length, or leaf area are altered in higher plants exposed to UV-B. Decrease in stem length, leaf area, and plant height by artificial or natural UV-B radiation was observed in cucumber, soybean, and sunflower (Sullivan and Teramura 1989, Tewini and Teramura 1989). UV-B radiation has many direct or indirect effects on plants including damage to DNA, proteins, and membranes, alterations in transpiration and photosynthesis, and changes in growth, development, and morphology (Whelan and Glaser 1997). The enhanced UV-B radiation inhibits photosynthetic activity through direct absorption by the leaves. Growth responses, particularly shoot elongation and leaf expansion, may be regulated through an action on the shoot tip presumably by destruction of growth hormones (Kulandaivelu et al. 1989). UV-B enhancement changes the growth of several plant species but does not reduce shoot dry mass (Barnes et al. 1990). UV-B exposure may also result in decreased contents of photosynthetic pigments, altered thylakoid integrity, increased stomatal diffusion, and reduced ribulose-1,5-bisphosphate carboxylase/oxygenase activity (Nogues and Baker 1995).

Even though photosynthetic pigments are bleached and disintegrated by UV-B radiation in marine phytoplankton (UNEP 1989), the vulnerable target in the photosynthetic systems was identified in aquatic plants (Häder et al. 1994). The role of epidermal pigments in protecting mesophyll photosynthesis of legumes (Shimazaki et al. 1988) and *Azolla microphylla* (Jayakumar et al. 1999) from UV-C radiation has been discussed. Many cyanobacteria have developed a number of adaptive strategies to reduce the adverse effects of excessive radiation. They include the avoidance of brightly irradiated habitats, the synthesis of UV screening pigments, and the production of chemical scavengers that detoxify the high-
ly reactive oxidants produced photochemically (Vincent and Roy 1993, Sinha et al. 1997, Zudaire and Roy 2001). Effects of UV radiation on aquatic plants and symbiotic systems have been scarcely reported (Häder and Figueroa 1997, Rozema et al. 1997, Jayakumar et al. 1999). Hence an attempt has been made to evaluate the influence of UV-B (280-320 nm) enhanced solar radiation in the vegetative growth, pigment contents, and photosynthetic activities in the aquatic fern *Azolla microphylla*.

**Materials and methods**

**Plants and growth conditions: Azolla microphylla**
Kauf. cultures were maintained in plastic troughs of 30 cm diameter and 20 cm depth. Filtered tap water with an electric conductivity of 0.7-0.8 mS cm\(^{-1}\) was used to grow the stock cultures. A mixture of 200 g wet land clay soil, 5 mg of superphosphate, and 50 g of fresh cow dung was placed in each trough. 4 000 cm\(^{2}\) of water was added to each trough to make slurry with the clay, superphosphate, and cow dung. The slurry was allowed to settle and to each trough 1 g of juvenile *Azolla* fronds was added. The water level was maintained at 10 cm above soil throughout the study period. Furadon (50 kg m\(^{-3}\)) was sprayed for pest control.

The troughs were placed under outdoor conditions in partial sunlight (UV-B of 300 mW m\(^{-2}\)). The average day/night temperature was 30/26±3 °C with an 11/13 h light/dark photoperiod.

**UV-B treatment: Azolla** cultures were exposed to UV-B enhanced cool fluorescent radiation for 30 min per d between 10.00 and 10.30 h from a sun lamp, Philips TL 20 W/12, placed at a distance of 30 cm above the troughs. The irradiance at sample surface was 1.5 W m\(^{-2}\) as measured by an IL 700 radiometer (International Light, USA).

**Experimental design:** The treatments were given at an interval of 3 d in the experimental period of 12 d. The fronds were used for analyses 24 h after irradiation and all experiments were replicated five times.

**Growth analysis:** Biomass, doubling time, relative growth rate (RGR) of *Azolla* fronds and heterocyst frequency of the symbiont *Anabaena* were determined in control and +UV-B irradiated plants. Doubling time and RGR were calculated by using the formula of Subudhi and Watanabe (1981):

\[ \text{doubling time [d]} = \frac{t}{r} \times 1, \]

**Results**

**Growth characteristics** (Table 1, Fig. 1): Biomass of control *Azolla* has increased by 15 fold due to growth during the experimental period of 12 d, while under UV-B radiation the growth decreased by 20 % over the control cultures. The doubling time under control and +UV-B was 3.08 and 3.35 d, respectively. This was also reflected in the RGR which was 0.207 kg kg\(^{-1}\) d\(^{-1}\) in +UV-B cultures showing a 9 % decrease from the control where \( t \) = experimental period, \( r = \log (M_1/M_0)/0.301, M_1 = \text{mass of the culture after } t \text{ d, } M_0 = \text{mass of the initial culture, } 0.301 = \text{constant.} \)

\[ \text{RGR [kg kg}^{-1} \text{ d}^{-1} \text{]} = 0.693 \text{ (doubling time)}^1 \]

Heterocyst frequency of control and UV-B irradiated *Azolla* bound *A. azollae* was calculated following the method of Kannaiyan and Kumar (1993).

**Pigment analysis:** Fresh *Azolla* fronds (including *Anabaena*) were extracted with 80 % acetone and the amounts of Chl and carotenoids were quantified according to Lichtenhaler and Wellburn (1983).

**PS2 electron transport system assay:** *Azolla* chloroplasts were isolated using the method of Kulandaivelu and Daniell (1980) and suspended in a medium containing 400 mM sucrose, 5 mM NaCl, and 25 mM HEPES-NaOH buffer (pH 7.8). The method of Noorudeen and Kulandaivelu (1982) was used to measure the rate of PS2 mediated electron transport in *Azolla* by using a Hansatech (U.K.) O\(_2\) electrode. The reaction mixture contained 20 mM Tris-HCl (pH 7.5), 5 mM MgCl\(_2\), 10 mM NaCl, 100 mM sucrose, 1 mM NH\(_4\)Cl, 1 mM BQ, and chloroplasts equivalent to 20 g(CHl) m\(^{-3}\).

**Chl a fluorescence kinetics:** In vivo Chl a fluorescence transients were followed in intact *Azolla* fronds after excitation with broadband blue radiation (400-620 nm, Corning CS 4-96) at a photon flux density of 460 μmol m\(^{-2}\)s\(^{-1}\) as described by Kulandaivelu and Daniell (1980). The fronds were kept in the dark at 28 °C for 5 min before fluorescence measurements. The signal from the photomultiplier was directly displayed either on a servo recorder (Hitachi model 056) or stored in a digital oscilloscope (Iwatsu SR 1100, Japan).
Table 1. Effect of UV-B enhanced radiation on biomass, doubling time, and relative growth rate (RGR) in *Azolla microphylla* (values are mean±SE of 5 samples).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>+UV-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass [g]</td>
<td>15.03 ± 1.10</td>
<td>11.99 ± 1.00</td>
</tr>
<tr>
<td>Doubling time [d]</td>
<td>3.08 ± 0.25</td>
<td>3.35 ± 0.20</td>
</tr>
<tr>
<td>RGR [kg kg(^{-1}) d(^{-1})]</td>
<td>0.225 ± 0.01</td>
<td>0.207 ± 0.01</td>
</tr>
</tbody>
</table>

Fig. 1. Changes in heterocyst frequency of *Azolla microphylla* fronds exposed to UV-B enhanced radiation.

**Pigment analysis** (Table 2): In the control, *Azolla* fronds showed an increase of 97% in total Chl content during the 12 d of growth. But under +UV-B such increase was only 54% for the same period. No marked change in Chl a/b ratio was observed. UV-B irradiation caused a 23% inhibition in carotenoid content on the 12th d after treatment.

Table 2. Changes in chlorophylls, Chl [g kg\(^{-1}\) (f.m.)] and carotenoids [mg kg\(^{-1}\) (f.m.)] of *Azolla microphylla* fronds exposed to UV-B enhanced radiation (values are mean±SE of 5 samples).

<table>
<thead>
<tr>
<th>Treatment [d]</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl a/b</th>
<th>Chl (a+b)</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.72 ± 0.20</td>
<td>0.77 ± 0.08</td>
<td>2.23 ± 0.21</td>
<td>2.49 ± 0.22</td>
<td>15.5 ± 2.0</td>
</tr>
<tr>
<td>3 Control</td>
<td>1.74 ± 0.10</td>
<td>0.80 ± 0.06</td>
<td>2.18 ± 0.20</td>
<td>2.54 ± 0.23</td>
<td>18.4 ± 1.5</td>
</tr>
<tr>
<td>+UV-B</td>
<td>1.69 ± 0.15</td>
<td>0.61 ± 0.05</td>
<td>2.77 ± 0.26</td>
<td>2.30 ± 0.20</td>
<td>14.0 ± 1.5</td>
</tr>
<tr>
<td>6 Control</td>
<td>2.58 ± 0.21</td>
<td>0.91 ± 0.08</td>
<td>2.83 ± 0.18</td>
<td>3.49 ± 0.31</td>
<td>20.2 ± 2.0</td>
</tr>
<tr>
<td>+UV-B</td>
<td>1.90 ± 0.15</td>
<td>0.73 ± 0.08</td>
<td>2.60 ± 0.19</td>
<td>2.63 ± 0.20</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td>9 Control</td>
<td>2.90 ± 0.15</td>
<td>1.05 ± 0.09</td>
<td>2.76 ± 0.25</td>
<td>3.95 ± 0.30</td>
<td>23.6 ± 1.1</td>
</tr>
<tr>
<td>+UV-B</td>
<td>2.39 ± 0.20</td>
<td>0.86 ± 0.07</td>
<td>2.78 ± 0.22</td>
<td>3.25 ± 0.28</td>
<td>18.4 ± 2.2</td>
</tr>
<tr>
<td>12 Control</td>
<td>3.60 ± 0.22</td>
<td>1.30 ± 0.10</td>
<td>2.77 ± 0.26</td>
<td>4.90 ± 0.41</td>
<td>26.4 ± 1.2</td>
</tr>
<tr>
<td>+UV-B</td>
<td>2.58 ± 0.21</td>
<td>0.97 ± 0.10</td>
<td>2.66 ± 0.25</td>
<td>3.55 ± 0.31</td>
<td>21.5 ± 1.5</td>
</tr>
</tbody>
</table>

**Discussion**

*Azolla* plants grown under UV-B enhanced solar radiation showed significant decrease in biomass, doubling time, RGR, and heterocyst frequency. These results agree with some earlier reports (Nouchi and Kobayashi 1995, Jayakumar et al. 1999). A very important symptom in UV-B treated *Azolla* plants is chlorosis and curling in the fronds (Jayakumar et al. 1999). Many sensitive higher plants grown under UV-B radiation in growth chambers, greenhouse, and field showed significant inhibition in growth and biomass (Nedunchezian and Kulandaivelu 1997, Lingakumar et al. 1999). Little is known about the environmental factors affecting doubling time, RGR, bio-
Table 3. Quantitative changes in $F_v/F_m$ and PS2 activity obtained from *Azolla microphylla* exposed to UV-B enhanced radiation (values are mean±SE of 5 samples).

<table>
<thead>
<tr>
<th>Treatment [d]</th>
<th>$F_v/F_m$ Control + UV-B</th>
<th>PS2 activity [mM(O$_2$) kg$^{-1}$ (Chl) s$^{-1}$] Control + UV-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.46 ± 0.03</td>
<td>256.2 ± 20.5</td>
</tr>
<tr>
<td>3</td>
<td>0.50 ± 0.04</td>
<td>275.5 ± 21.3</td>
</tr>
<tr>
<td>6</td>
<td>0.53 ± 0.04</td>
<td>367.0 ± 15.6</td>
</tr>
<tr>
<td>9</td>
<td>0.56 ± 0.05</td>
<td>372.0 ± 15.6</td>
</tr>
<tr>
<td>12</td>
<td>0.43 ± 0.04</td>
<td>380.9 ± 25.2</td>
</tr>
</tbody>
</table>

mass, and heterocyst frequency of *Azolla*. UV-B radiation inhibits vegetative growth, which is attributed to the reduction in the synthesis of auxin derivatives in higher plants and in *Azolla* fronds (Kulandaivelu et al. 1986, Jayakumar et al. 1999). IAA effect may be one of the factors which stimulate the growth of *Azolla* (Dusek and Bonde 1965). The decrease in biomass, RGR, and symbiotic heterocyst frequency of *Azolla* fronds may be due to the disturbance in IAA synthesis induced by UV-B radiation (values not shown).

The Chl and carotenoids contents in control and UV-B treated *Azolla* fronds showed a small increase during the 12 d growth period. Earlier reports indicate that UV-B irradiation during growth produces significant reduction in Chl content (Lingakumar and Kulandaivelu 1998, Jayakumar et al. 1999). Enhanced UV-B radiation causes damage to Chl $a$ and Chl $b$ due to inhibition in Chl biosynthesis (El-Mansy and Salisbury 1971). As *Azolla* fronds possess only two or three layers of mesophyll tissue (Lumpkin 1987), the photosynthetic pigments have been bleached upon UV-B treatment.

UV-B induced changes on the photosynthetic electron transport chain were reported by Nedunchezian and Kulandaivelu (1991, 1997) and Jayakumar et al. (1999). UV-B induced inhibition of photosynthetic activity was demonstrated in some marine and freshwater cyanobacteria (Sinha and Häder 1996). Among all the target sites, PS2 is probably the most important site for UV-B. The significant increase in the rate of O$_2$ evolution in the control *Azolla* fronds from 0 to 12 d may be due to either the efficient functioning of primary photochemistry or to an increase in the number of PS2 units per chloroplast.

In the UV-B treated *Azolla* fronds the increase in the rate of O$_2$ evolution was observed only from 3$^{rd}$ to 9$^{th}$ d and thereafter declined rapidly to about 40% of the respective control rate. The concentration of Chl pigments in the UV-B treated fronds demonstrated only an increase till the 12$^{th}$ d and thus it seems to be not affected by UV-B exposure. Therefore the decrease in PS2 activity might be due to the damage in the PS2 protein complex. This is in agreement with reports of Nedunchezian and Kulandaivelu (1997) and Jayakumar et al. (1999). The correlation between the UV-B induced degradation of D$_1$ protein and the presence of functional Mn on the donor and/or acceptor side of PS2 was reported by Barbato et al. (1995). They suggested that this region of PS2 is a target for UV-B radiation.

Fluorescence induction curves reflect photosynthesis and electron transport. They have characteristic patterns, which change when the photosynthetic systems become damaged. The fast fluorescence transients show a typical O, I, D, and P pattern (Govindjee and Papageorgiou 1971). *Azolla* fronds exposed to UV-B radiation showed a reduction in variable (I-P) fluorescence yield. This indicates the damage of PS2 activity, particularly at the donor site (Kulandaivelu et al. 1989). Changes in fluorescence parameters were observed also in mosses and lichens (Csintalan et al. 2001) or bean (Kreslavski et al. 2001).

All the above experiments show that enhanced UV-B radiation inhibits the growth of *Azolla*, which may be due to the destruction of plant growth hormones and photosynthetic activity, through direct absorption by the fronds.

References


ENHANCED ULTRAVIOLET-B (280-320 nm) EFFECTS ON AZOLLA


