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Changes in pigment composition and photosynthetic activity of aquatic fern (*Azolla microphylla* Kaulf.) exposed to low doses of UV-C (254 nm) radiation

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Abstract

Changes in the content of pigments and rate of photosynthesis in *Azolla microphylla* Kaulf. fronds were measured during growth under solar and ultraviolet-C (UV-C) supplemented solar radiation. Maximum content of total chlorophyll (Chl) was observed on the 13th day (termination of the experiment) of treatment in both control and treated plants. The treated plants had significantly lower total Chl and carotenoid contents than the control plants during the 1st day of growth. After the 4th day of exposure to UV-C supplemented solar radiation, the Chl and carotenoids accumulation increased in treated plants, so that the pigment concentration in the treated fronds was nearer to the control values after the 13th day of treatment. Significant increase in UV absorbing pigments, anthocyanins, and flavonoids was observed at the 13th day of treatment. In spite of the roughly similar photosynthetic pigment concentration, the photosynthetic activity measured as the rate of electron transport at photosystem 2 was only 65 % of the control values after 13 d of UV-C exposure.

Additional key words: anthocyanin; carotenoids; chlorophyll; flavonoids; photosystem 2.

Introduction

UV radiation affects growth and photosynthesis in phytoplankton algae, cyanobacteria, and some marine plants (Döhler and Alt 1989, Wilhelm *et al.* 1997). UV radiation adversely affects the function of photosystem 2 (PS2) in chloroplasts (Van *et al.* 1977, Kulandaivelu and Noorudeen 1983, Nedunchezhian and Kulandaivelu 1991, Biswal *et al.* 1997, Lingakumar and Kulandaivelu 1998).

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Abbreviations: DCPIP - dichlorophenol indophenol; PS2 - photosystem 2; UV-C - ultraviolet-C radiation (<280 nm).

Even though photosynthetic pigments are bleached and destroyed by UV radiation in the marine phytoplankton (UNEP 1989), the vulnerable target in the photosynthetic systems has not been clearly identified in aquatic plants. Increase in synthesis of UV-absorbing or UV-shielding pigments such as anthocyanins and flavonoids is the general protective mechanism in plants exposed to UV radiation. The role of epidermal pigments in protecting mesophyll photosynthesis of legumes from UV-C radiation has been discussed by Shimazaki *et al.* (1988). In contrast to higher plants (*e.g.*, Moorthy and Kathiresan 1997), very few phytoplanktons and cyanobacteria reportedly produce UV absorbing or UV-B inducible pigments (Garcia-Pichel and Castenholz 1991).

Azolla, the aquatic fern with its nitrogen fixing symbiont *Anabaena azollae*, has been used as a biofertilizer for lowland rice. Effects of UV radiation on aquatic plants and symbiotic systems have been scarcely reported. This paper reports the influence of UV-C (<280 nm) supplemented solar radiation on the concentration of photosynthetic and UV-shielding pigments and on the photosynthetic activity of *Azolla microphylla*.

Materials and methods

Plants: *A. microphylla* Kaulf. cultures were maintained in plastic troughs of 30 cm diameter and 20 cm depth. Filtered tap water with an electric conductivity of 0.8 mmhos cm^{-1} was used for growing the stock cultures. A mixture of 200 g wet land clay soil, 5 mg of superphosphate, and 50 g of fresh cow dung were placed in each trough. 4 000 cm^3 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 fronds of *Azolla* was added. The water level was maintained at 10 cm above soil throughout the study period. In addition, furadon (50 g m^{-3}) was given as spray for pest control.

The troughs were placed under outdoor conditions in partial sunlight in the greenhouse of the VHNSN College, Virudhunagar. The average day/night temperature was 32/26 °C. The natural photoperiod varied between 10 and 12 h.

UV-C treatment: *Azolla* culture received UV-C (<280 nm) irradiation for 30 min per d between 10:00 and 10:30 h from a UV lamp placed at a distance of 30 cm above the troughs. A *Philips* germicidal lamp *TUV* 30 W was used as UV-C source. The irradiance at sample surface was 13 W m^{-2} as measured by an *IL 700* radiometer (*International Light*, USA) with the detector *PT 171 C*.

Experimental design: All experiments were replicated five times. The plants were used for analysis 24 h after irradiation and subsequently at an interval of three days till the 13th day. Photosynthetic measurements were performed on the 1st and 13th day.

Estimation of pigments: *Azolla* fronds were extracted with 80 % acetone and the contents of Chl and carotenoids were estimated according to Lichtenthaler and Wellburn (1983). For estimation of anthocyanins and flavonoids, *Azolla* fronds were soaked in 10 cm^3 of acidified methanol (methanol : water : HCl, 80 : 20 : 1, v/v/v) and left overnight at –20 °C. The absorbance of the clear extract was measured at 657, 530, and 315 nm (using a *Hitachi 557* spectrophotometer). Contents of anthocyanins (Mancinelli *et al.* 1975) and flavonoids (Mirecki and Teramura 1984) were calculated using the following formulae:

$$\text{Anthocyanins [g kg}^{-1}] = \frac{(A_{530}) - (0.3\ A_{657})\ V\times 1000}{M}$$

$$\text{Flavonoids [g kg}^{-1}] = \frac{(A_{315})\ V\times 1000}{M}$$

where V is volume of the extract [cm^3], and M is fresh mass of the sample [g].

Photosynthetic activity: *Azolla* chloroplasts prepared as described by Kulandaivelu and Daniell (1980) were suspended in a medium containing 400 mM sucrose, 5 mM NaCl, 5 mM $MgCl_2$, and 25 mM HEPES-NaOH buffer, pH 7.8. The rate of PS2 mediated electron transport in *Azolla* was determined spectrophotometrically (*Hitachi 557*, Japan) by measuring the reduction of DCPIP at 580 nm following the method of Fleishhacker and Senger (1978). The reaction mixture contained 25 mM HEPES-NaOH buffer, pH 7.5, 5 mM $MgCl_2$, 5 mM NaCl, 5 mM NH_4Cl, 0.1 M sucrose, 50 mM DCPIP (freshly prepared), and chloroplasts equiavalent to 20 μg Chl.

Results and discussion

The biomass of *Azolla* plants exposed to UV–C radiation was 29% less than that of the control plants on the 13th day of treatment. Consequently doubling time increased from 10 to 16 d in the treated plants indicating a decrease in the relative growth (Table 1).

The Chl content of *Azolla* during the 13 d-growth of controls showed only a small increase (Fig. 1). UV-C treatment caused a 48 % decrease after one day of treatment. Such photobleaching of Chl due to UV exposure has been widely reported. Penetration of UV-B radiation depends on the nature of leaves. Herbaceous dicots showed 18-41 % epidermal transmittance (UNEP 1989). *Azolla* fronds having only two or more layers of mesophyll tissue (Lumpkin 1987) are likely to get their pigments bleached upon UV-C treatment. The much lower Chl content in UV-C treated plants after a day exposure is expected in the absence of UV induced synthesis of protective pigments. After 4 d of UV-C treatment the total Chl content of treated plants increased with a higher rate, and after 13 d of UV-C exposure the pigment concentration was 82 % of the respective control values. This indicates an increased synthesis of protective pigments and of turnover processes (Fig. 1). The results agree with those of Teramura (1983) who observed that carotenoids are less affected by UV-B radiation than Chl in soybean seedlings.

Table 1. Effect of UV-C radiation on biomass, doubling time, and relative growth rate in *Azolla microphylla* (values are means ± SE of 5 samples).

Parameter	Control	+UV-C
Biomass [g]	2.40 ± 0.20	1.70 ± 0.15
Doubling time [d]	10.40 ± 1.00	16.40 ± 1.40
Relative growth rate [kg kg^{-1} d^{-1}]	0.07 ± 0.005	0.04 ± 0.005

The content of the UV shielding pigments was increased in *Azolla* fronds after 13 d of UV-C treatment. The anthocyanin and flavonoid contents have increased by 18.5 and 3.7 %, respectively, with reference to control plants (Fig. 1). Large increase in the content of flavonoids was observed in both control and UV-C treated plants. The content of flavonoids in UV-C exposed plants was always higher than in the control and the difference was larger during the early period of treatment. Similar to this, the anthocyanin content of treated plants was also higher than in the control plants throughout the treatment period. Braun (1991) reported that the epidermal layer of oat seedlings accumulated large amounts of UV-absorbing pigments during early development which gave a better protection against UV-B than in rye seedlings. UV-B induced increase in flavonoids was also demonstrated in two species of *Aquilegia* growing at different altitudes.

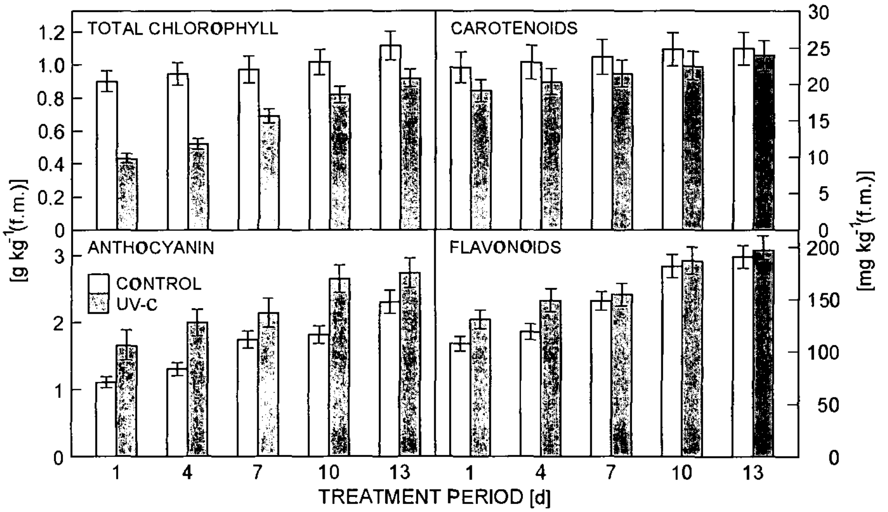

Fig. 1. Changes in total chlorophyll, carotenoid, anthocyanin, and flavonoid contents in the *Azolla microphylla* fronds from 1st to 13th day after the onset of UV-C treatment.

Though the initial decrease in the photosynthetic pigments was leveled off to reach the control values on the final day of irradiation, the photosynthetic activity measured as the PS2 electron transport ($H_2O \rightarrow$ DCPIP) was only 74 % after a day of exposure (Table 2).

Table 2. Effect of UV-C radiation on photosynthetic activity [mmol(DCPIP reduced) kg^{-1}(Chl) s^{-1}] in *Azolla microphylla* (values are means + SE of 5 samples).

Treatment period [d]	Control	+UV-C
1	27.4 ± 2.8	20.2 ± 1.8
13	39.4 ± 3.0	13.2 ± 1.0

Because the total nitrogen requirement of *Azolla* fronds is supplied by the algal symbiont *A. azollae*, UV-C induced damage to the nitrogen fixation process might inhibit photosynthetic productivity indirectly through the proteins of photoreceptor and photosynthetic apparatus. Since *Azolla* depends on its algal symbiont for the total nitrogen requirement, UV-C induced damage to the nitrogen fixation process (Kulandaivelu 1989) would affect the biosynthesis of the proteins. Thus the photosynthetic productivity of *Azolla* may be inhibited indirectly by its algal symbiont. This necessitates further studies of the UV-C induced damage to the nitrogen fixation process.

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