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BRIEF COMMUNICATION

Responses of nine bryophyte and one lichen species from different microhabitats to elevated UV-B radiation

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Abstract

Chlorophyll fluorescence parameters (F_v/F_m, R_{Fd}) of nine bryophyte and one lichen species were investigated after prolonged exposure to elevated UV-B radiation. The majority of the investigated bryophytes showed a prompt or inducible tolerance to increased UV-B irradiation. Among the investigated species high degree of UV-tolerance coincides with strong desiccation tolerance.

Additional key words: chlorophyll fluorescence induction; desiccation tolerance; fluorescence emission spectrum.

Responses of plants to different UV-treatments have been widely investigated. About a half of the *ca.* 600 species tested proved to be sensitive (Teramura 1998). Most researchers were concerned about crop plants and we could only find one experiment dealing with mosses (Björn *et al.* 1997). The primary importance of bryophytes lies not within their biomass production but rather in their ability to modify their environment in terms of microclimate, water economy, soil characteristics, and decomposition rate (Oechel and Lawrence 1985). Therefore it becomes crucial to map their sensitivity to the shifting global environmental factors. On the other hand, desiccation tolerance is abundant among bryophytes. This tolerance against extreme drought stress might make easier for them to cope with other stress factors, *e.g.*, UV-B radiation.

Most articles deal with the damage of the photochemical apparatus, *i.e.*, the water oxidising side of the PS2 in response to UV-B radiation (Kulandaivelu *et al.* 1991, Vass *et al.* 1996). Reduced ribulose-1,5-bisphosphate carboxylase/oxygenase concentration and activity may also results from the harmful effects of UV-B (Jordan *et al.* 1992). UV-B effects may also be similar to those induced by deficiency of mineral elements important for photosynthesis, such as Mg (Premkumar and Kulandaivelu 1999).

Some of the UV-effects are exerted through evoking oxidative stress. The evolved oxidative radicals cause membrane disruption and electrolyte leakage (Dai *et al.* 1997). UV-B radiation generates hydroxyl and carbon centred radicals (Hideg and Vass 1996). Desiccation, even in desiccation tolerant plants, induces oxidative stress (Dhindsa and Matowe 1981, Smirnoff 1993). Therefore, the biochemical backgrounds of the two stress factors may overlap.

Several vascular plants cope with increasing UV-B radiation through accumulating UV-filtering flavonoids within the epidermis (Bornmann and Teramura 1993, van de Staaij *et al.* 1995). Lichens contain a high amount of secondary metabolites (Lawrey 1995), but UV-B radiation can increase it further (Fahselt 1994). Bryophytes can synthesise flavones and flavanols (Stafford 1991), hence this adaptation strategy must be investigated in bryo-

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Abbreviations: Chl – chlorophyll; F_0 – minimal level of fluorescence; F_m – maximum level of fluorescence; F_v – variable fluorescence; F_v/F_m – maximum photochemical efficiency of photosystem 2; F_s – steady-state fluorescence of light-adapted leaves; F_{450}, F_{520}, F_{690}, F_{735} – fluorescence intensities determined at 450, 520, 690, and 735 nm, respectively; PAR – photosynthetically active radiation; PS2 – photosystem 2; R_{Fd} – fluorescence decrease ratio; UV-B – ultraviolet radiation (280–320 nm).
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phytes as well. Peaks in the blue and green regions (440 and 520 nm) of the UV-induced fluorescence emission spectrum reflect secondary phenolic metabolites located and covalently bound to the cell walls. The characteristic ratios of fluorescence bands (F_{440}/F_{690}, F_{440}/F_{735}, F_{440}/F_{520}, F_{690}/F_{735}) are suitable for early detection of senescence, water deficit, UV-stress, heat stress, viral and fungal infections, *etc.* (Lichtenthaler *et al.* 1991, Middleton *et al.* 1996, Schweiger *et al.* 1996, Lichtenthaler and Schweiger 1998) and desiccation/rehydration (Takács *et al.* 2000). In this study we screened nine bryophyte species to reveal if bryophytes will be affected in the future by high UV-B environment.

Eight bryophyte species were collected in the Ardennes, Belgium: the mosses *Plagiothecium undulatum* (Hedw.) Br. Eur., *Leucobryum glaucum* (Hedw.) Angstr., *Polytrichum formosum* Hedw., *Mnium hornum* Hedw., *Dicranum scoparium* Hedw., *Plagiomnium undulatum* (Hedw.) Kop., *Sphagnum capillifolium* (Ehrh.) Hedw., and the liverwort *Pellia epiphylla* (L.) Corda. The moss *Tortula ruralis* (Hedw.) Gaertn. *et al.* ssp. *ruralis* and the lichen *Cladonia convoluta* (Lam.) P. Cout. grew in a semi-arid sandy grassland in Hungary (Kiskunsági National Park). The test plants were kept in a greenhouse without additional PAR irradiation; they were continuously hydrated to avoid desiccation because its effects could not have been separated from those caused by UV-radiation.

UV-treatment was provided by UV-B fluorescent tubes (*Philips TL 12*/40 W). We used 95 μm cellulose diacetate filters (*Clarifoil, Courtaulds*) in a frame hanging between tube and plants in order to exclude radiation below the wavelength 280 nm. The filters were replaced every third day. Supplying UV-B (1.286±0.018 W m^{-2})—unweighted radiation—for 4 (low UV-B treatment) or 8 (high UV-B treatment) h a day resulted in a daily dose of 18.52 and 37.04 kJ m^{-2} d^{-1}, respectively. The exposure lasted for 34 d, thereafter UV-B was shut down and chlorophyll (Chl) fluorescence was measured once a week. For fluorescence spectra measurements, *T. ruralis* and *C. convoluta* were kept in a plant growth chamber experiencing 10 h of photoperiod (μmol m^{-2} s^{-1}) every day. UV-B treatment (1.86 W m^{-2}) was given 8 h a day (53.57 kJ m^{-2} d^{-1}). During the six days of exposure plants were kept at optimum water content.

The effects of UV-B on bryophytes were monitored by Chl fluorescence kinetics measurement before the 4- or 8-h-long daily treatments. Both fast and slow phases of the fluorescence kinetics of dark adapted (for 25 min) samples were measured. The fluorescence induction kinetic curves were recorded by the *Morgan CF1000* (USA) fluorometer. The efficiency of the primary photochemistry of photosystem 2 (PS2), as given by the ratio F_v/F_m $[(F_m - F_0)/F_m]$ and the variable fluorescence decrease ratio $[R_{Fd} = (F_m - F_s)/F_s]$ referring to the potential thylakoid membrane activity were measured as described by Csintalan *et al.* (1999). UV-induced fluorescence emission spectra were recorded using a *Perkin-Elmer LS-50* Luminescence Spectrometer (*Perkin-Elmer*, Übelingen, Germany) (Schweiger *et al.* 1996). The fluorescence yield between the wavelengths 400 and 800 nm was recorded at an excitation wavelength of 340 nm.

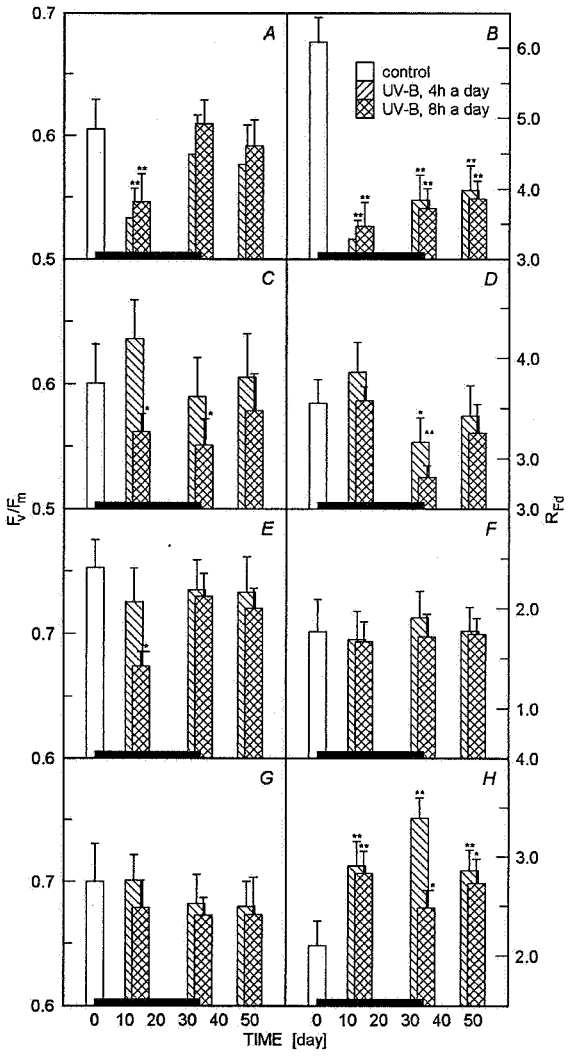

Fig. 1. F_v/F_m (*left*) and R_{Fd} (*right*) values (means of 5 replicates) of *Plagiothecium undulatum* (*A, B*), *Leucobryum glaucum* (*C, D*), *Polytrichum formosum* (*E, F*), and *Dicranum scoparium* (*G, H*) after two and four weeks of UV-B treatment (1.286±0.018 W m^{-2} for 4 or 8 h a day) and two weeks of recovery afterwards. The dark horizontal line represents the duration of the UV-B treatment period. Asterisks denote the significant deviation from control values ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$).

The fluorescence parameter F_v/F_m was relatively insensitive to UV-B radiation. A slight but lasting decrease was found in *S. capillifolium*. *P. undulatum* and *M. hornum* (Fig. 1*A*) suffered temporal damage, but they could adapt to the low UV-B dose. Low UV-B radiation did not

alter F_v/F_m in other species. Nevertheless, high UV-B dose had a greater impact on F_v/F_m values: *L. glaucum* (Fig. 1*C*) and *P. epiphylla* recovered only after the end of the treatment. *P. undulatum*, *M. hornum*, and *P. formosum* (Fig. 1*E*) became soon accustomed to UV-B treatment after a transient fall. In terms of photochemical efficiency of PS2, the other species were still resistant (*e.g.*, Fig. 1*G*).

Fluorescence decrease ratio showed more dramatic changes due to the UV-B treatment. Both UV-B treatments caused a permanent decrease in *P. undulatum* (Fig. 1*B*), *P. epiphylla*, and a smaller but also steady one in *P. undulatum*. *L. glaucum* (Fig. 1*D*) could recover only after UV-irradiation had been finally stopped. R_{Fd} values of *P. formosum* (Fig. 1*F*), *M. hornum*, *T. ruralis*, and *S. capillifolium* were insensitive to either UV-B doses. *D. scoparium* (Fig. 1*H*) revealed a stimulation in response to the UV-B treatments. However, this increase was not sustained at the high UV-level. After turning off the UV-radiation, the stimulation seemed to decay.

In some species (*P. formosum*, *M. hornum*, *S. capillifolium*) only F_v/F_m decreased. We suggest that in dark-adapted plants the original thylakoid membrane activity can be kept up with a slightly reduced number of active PS2 reaction centres. Only *S. capillifolium* experienced a sustained decrease in F_v/F_m without R_{Fd} loss. Further studies are needed to make a hypothesis concerning the UV-responses of this unique taxonomic group.

The grassland moss *T. ruralis* is characterised by the greatest extent of desiccation tolerance (Tuba *et al.* 1996). This species failed to respond to the UV-B treatment at all.

Desiccation tolerant forest mosses (such as *P. formosum* and *M. hornum*) are able to acclimate even to the higher UV-B dose. Their PS2 activities, measured by F_v/F_m, decreased only temporarily. The up-regulation of either protective or repair mechanisms can explain this phenomenon. *D. scoparium*, another desiccation tolerant forest moss, behaved different from the afore-mentioned two. Its PS2 reaction centres showed full functionality as measured by F_v/F_m. On the other hand, R_{Fd} increased as a response to both UV-treatments. This stimulation was transient under the higher dose, but the lower one seemed to be advantageous in the long run. The stimulation effect is in accord with the findings of Björn (1997) who reported an increased stem growth rate in response to elevated UV-B irradiation.

P. undulatum, which inhabits rather shaded habitats, can also repair PS2 reaction centres. On the other hand, its R_{Fd} value, reflecting overall photosynthetic activity, declined fast and could not recover. This supports the view that sensitive parts other than PS2 are within the photosynthetic system. *P. undulatum* is also a mesic species and shows a similar response to UV-B radiation.

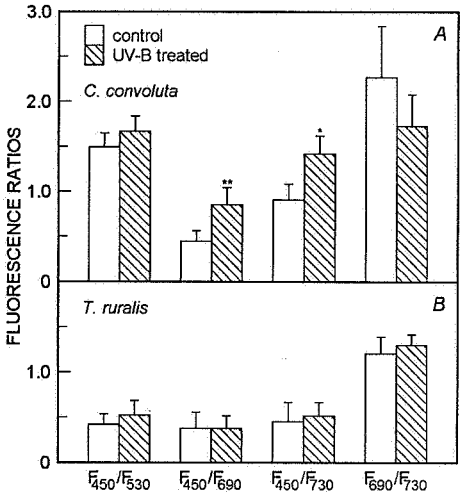

Fig. 2. Ratios of characteristic fluorescence bands in the case of *Cladonia convoluta* (*A*) and *Tortula ruralis* (*B*) after six days of UV-B treatment (53.57- kJ m^{-2} d^{-1}). Asterisks denote the significant deviation from control values ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$).

L. glaucum thrives in marshlands and other wet habitats. Repair mechanisms in this species cannot keep up with damage and PS2 activity can recover only after the cessation of UV-B radiation. The decrease in F_v/F_m was prompt due to the high UV-B dose, while there was a lag time before the fall in R_{Fd} appeared.

The desiccation intolerant liverwort *P. epiphylla* was the most sensitive to UV-B. Though it could repair PS2 centres after turning off the UV-radiation, the overall photosynthetic activity decreased by 50 % irreversibly.

The blue/red and blue/far-red fluorescence ratios of *C. convoluta* (Fig. 2*A*) increased in response to UV-B-treatment, suggesting the additional synthesis of blue-fluorescing secondary metabolites. As a contrast to the lichen species we could not detect the same in *T. ruralis*. This finding and the immediate and full tolerance to extremely high UV-B of this species (63.36 kJ m^{-2} d^{-1} UV-B radiation, values not shown) exclude the possibility that UV-inducible screening molecules would play a key role in the tolerance mechanism of mosses.

Hence the majority of the bryophytes investigated showed a prompt or inducible tolerance to elevated UV-B radiation. Among the investigated species elevated UV-tolerance coincides with high desiccation tolerance.

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