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UV-B radiation and selenium affected energy availability in green alga *Zygnema*

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Abstract: Green alga *Zygnema* was exposed to three concentrations of selenium and two levels of UV-B radiation. The combined effects of both treatments on energy availability; photochemical quantum yield and respiratory potential were studied. Our findings show that traces of selenium enhance metabolic process connected with photochemical quantum yield and mitochondrial respiration. Surprisingly, selenium does not diminish the effects of UV-B radiation; on the contrary, the combined action of UV-B radiation and traces of selenium leads to pronounced negative effects on photochemical quantum yield and the respiratory potential. Selenium is involved in the activation of energy resources in green alga *Zygnema*. The importance of selenium for activity of the mitochondria is possibly an evolutionary recollection from an endosymbiotic bacterium.

Key words: electron transport system; PSII; selenium; UV radiation; *Zygnema*

Introduction

Selenium (Se) is of metabolic importance in cyanobacteria (Li et al. 2003) and in some plants, being involved in their antioxidative processes (Harrison et al. 1988; Ekelund & Danilov 2001; Fu et al. 2002; Seppänen et al. 2003). Se is widely distributed on the Earth's surface and available for plants in at least small traces. However, some regions like Slovenia, suffer from a relative deficiency of Se (Kreft et al. 2002). Se is known as a trace element, essential in very small quantities for antioxidative functions in higher animals and humans. Whether it is essential for higher plants and involved in their metabolism is poorly understood. There is evidence for its involvement in antioxidative processes in higher plants (Seppänen et al. 2003); as selenocysteine in the glutathione peroxidase homologue in *Chlamydomonas* (Fu et al. 2002), and in protecting *Euglena* against UV-B radiation stress (Ekelund & Danilov 2001). Thus it could be important in mitigating the effects of increasing UV-B radiation at the Earth's surface, which is harmful to organisms.

The deleterious effects of UV-B on plants include damage of molecular targets, causing disturbance to photosynthetic carbon fixation (Hazzard et al. 1997; Rozema et al. 1997; Björn 1999), and consequently changing the community structure and productivity of aquatic ecosystems (Mittler & Tel-Or 1991; Häder et al. 2001). Various mechanisms mitigating UV damage have evolved in phototrophic organisms. The most important are excision repair, photoreactivation (Eker et al. 1990), the accumulation of detoxifying enzymes for eliminating UV-induced oxygen radicals (Mittler & Tel-Or 1991) and the production of UV absorbing compounds (Karsten et al. 1999; Yao et al. 2005). Establishing protective and repair mechanisms is costly in energy (Cullen & Nealle 1994).

Chlorophyll fluorescence is a very informative indicator of primary photosynthetic electron transport in PSII (Schreiber & Bilger 1992). The optimal quantum yield of PSII (Fv/Fm) quantifies its potential photochemical efficiency (Schofield et al. 1995). Any deviation from the optimal quantum yield can be used as an integrative measure of stress to which a plant is exposed (Schreiber et al. 1995). Measurement of electron transport system (ETS) activity of mitochondria is a good tool for estimating the respiratory potential of organisms (Töth 1993).

This study was aimed to examine the effects of the addition of Se traces to the growth medium on primary and terminal electron transport activity, and thus the availability of energy in green alga *Zygnema* sp. from Alpine Lake Kriško Inferior, exposed to two levels of UV-B radiation; high dose (7.4 UV-B_{BE} kJ m^2/day), and low dose (1.1 UV-B_{BE} kJ m^2/day).

Material and methods

Plant material and growth conditions

Filamentous alga *Zygnema* sp. was collected in a mountain lake, Kriško Inferior (altitude 1880 m, N 46°24′32″,

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E 13°48′34″) in NW Slovenia, about 0.5 m under the water surface. Algae were cultured in a growth chamber at 20 ± 2 °C under 200 µmol m^{-2} s^{-1} photosynthetic active radiation (PAR), immediately after bringing from the lake, for 24 hours before the measurements (12/12 h light/dark). Q-Panel UV-B 313 fluorescent lamps were used as a UV-B source. Lamps were wrapped in Mylar foil to eliminate wavelengths below 320 nm (Gehrke et al. 1996) for low UV-B dose (UV-B (L)), and in cellulose diacetate filters to eliminate UV-C radiation (lower than 290 nm) for high UV-B dose (UV-B (H)). Sufficient aeration was provided to exclude possible phytotoxic effects of cellulose diacetate (Krizek & Mirecki 2004). The low and high UV-B doses corresponded to 1.11 and 7.4 kJ m^2/day of UV-B$_{\mathrm{BE}}$ respectively, using the generalized plant action spectrum (Caldwell 1968). The doses applied were comparable to the summer (UV-B (H)) and winter (UV-B (L)) doses, measured in Slovenia in the last years (Gaberščik et al. 2002). UV-B radiation and PAR were measured at the surface level of the algal suspension using the ELDONET dosimeter (European Light Dosimeter Network). Algae were exposed to two levels of Se concentration 24 hours before measurements. UV-B radiation treatment was performed for 6 hours in the beginning of 12 h light period. Six different treatments were applied: UV-B(L), UV-B(L) + Se-1, UV-B(L) + Se-2 and UV-B(H), UV-B(H) + Se-1, and UV-B(H) + Se-2. Se-0 was natural lake water, with 10% addition of distilled water, and did contain any detectable amount of Se; Se-1 and Se-2 was the same lake water with 10% addition of distilled water containing Na selenate, to give final concentrations 0.001 mg Se/L (Se-1) and 0.1 mg Se/L (Se-2) in the growth medium.

Fluorescence measurements

The quantum yield of PSII was measured using a fluorometer OS-500 (Opti-Sciences, Tyngsboro, MA, USA). The optimal quantum yield expressed as Fv/Fm, is a measure of the efficiency of energy conversion in PSII. Fv is a variable fluorescence, Fo and Fm are minimal and maximal chlorophyll *a* fluorescence yield in dark adapted sample; Fv = Fm-Fo. Algae were kept in dark for 15 min before measurement at ambient temperature. Fluorescence was excited with a saturating beam of "white light" (PPFD = 8 000 µmol m^{-2} s^{-1}, 0.8 s). The effective quantum yield of PSII gave the actual efficiency of energy conversion in PSII (Björkman & Demmig-Adams 1995). It was measured under saturating irradiance at the prevailing ambient temperature by providing a saturating pulse of "white light" (PPFD = 9 000 µmol m^{-2} s^{-1}, 0.8 s) using a standard 60° angle clip. The effective quantum yield of PSII was defined as yield = (Fm'-F)/Fm'= ΔF/Fm'. Fm' is maximal fluorescence of an illuminated sample and F is steady state fluorescence (Schreiber et al. 1995*).*

Measurement of terminal electron transport system activity

Respiratory potential was estimated *via* the terminal electron transport system (ETS) activity of mitochondria as described by Packard (1971) and modified by Kenner & Ahmed (1975). Algae were homogenized in cold buffer (0 °C, 0.1 M sodium phosphate buffer pH 8.4, 75 µM MgSO$_4$, 0.15% (w/v) polyvinyl pyrrolidone, 0.2% (v/v) Triton-X-100) in a mortar and with an ultrasound homogeniser (4710; Cole-Parmer, Vernon Hills, IL, USA) and centrifuged (8,500 *g* at 0 °C for 4 min) in a top refrigerated ultracentrifuge. The supernatant was mixed with substrate solution (0.1 M sodium phosphate buffer pH = 8.4, 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100), and 20 mg 2-p-iodo-phenyl 3-p-nitrophenyl 5–phenyl tetrazolium chloride (INT) in 10 ml of bidistilled water; the assay mixture was incubated for 40 min at 20 °C. INT was used as the electron acceptor, being reduced to formazan, whose absorbance is measured at 490 nm. ETS activity was calculated from the rate of INT reduction, which was converted to the amount of oxygen utilized per g of alga dry mass (DM) per hour.

Statistical analysis

Measurements were carried out on 4 to 8 parallel samples. The significance of differences between treatments was tested by ANOVA (Statgraphics Version 4, Statistical Graphics Corp.).

Results

It follows from Fig. 1A that neither UV-B irradiation nor Se, either singly or together, affected Fv/Fm.

The higher Se concentration in the growth medium, 0.1 mg Se/L (Se-2), considerably increased the effective quantum yield under low (UV-B (L)) radiation dose (Fig. 1B). The effect of Se on high UV-B dose (UV-B (H)) treated algae was opposite to that on algae exposed to UV-B (L) dose. Se, at concentrations of 0.001 mg Se/L (Se-1) and 0.1 mg Se/L (Se-2), lowered the effective quantum yield of UV-B (H) irradiated algae. In the absence of Se there was no difference in the effects

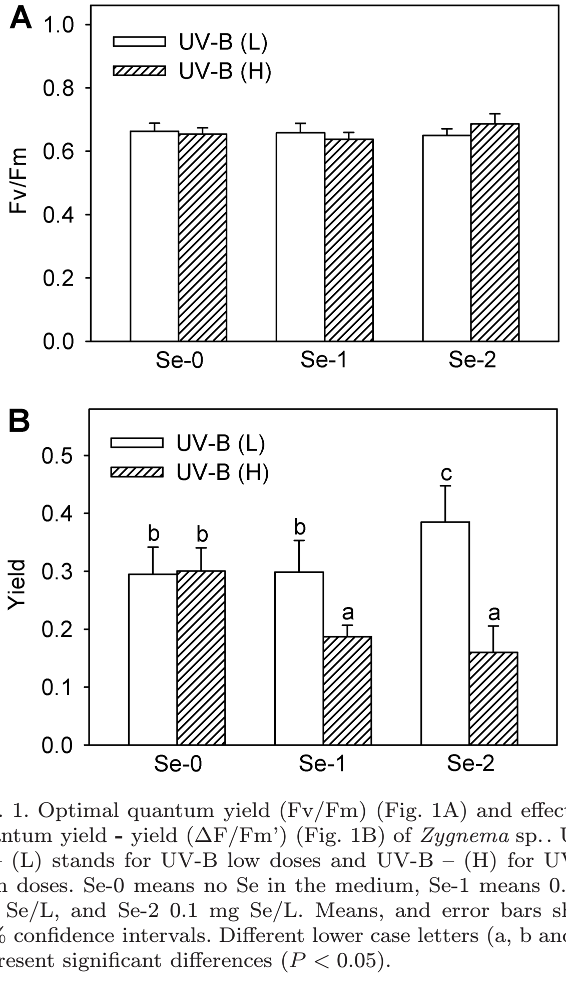

Fig. 1. Optimal quantum yield (Fv/Fm) (Fig. 1A) and effective quantum yield - yield (ΔF/Fm') (Fig. 1B) of *Zygnema* sp.. UV-B – (L) stands for UV-B low doses and UV-B – (H) for UV-B high doses. Se-0 means no Se in the medium, Se-1 means 0.001 mg Se/L, and Se-2 0.1 mg Se/L. Means, and error bars show 95% confidence intervals. Different lower case letters (a, b and c) represent significant differences ($P < 0.05$).

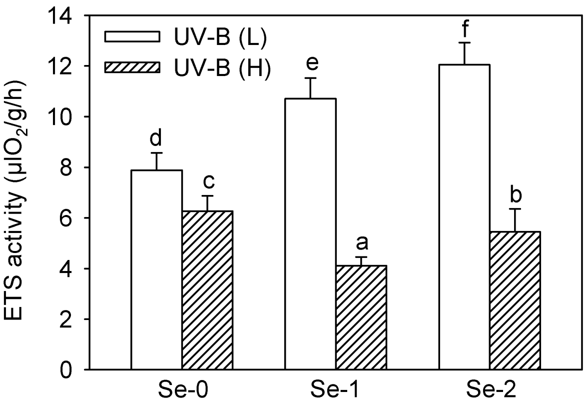

Fig. 2. Terminal electron transport system (ETS) activity per dry mass (DM) of *Zygnema* sp. UV-B – (L) stands for UV-B low doses and UV-B – (H) for UV-B high doses. Se-0 means no Se in the medium, Se-1 means 0.001 mg Se/L, and Se-2 0.1 mg Se/L. Means, and error bars show 95% confidence intervals. Different lower case letters (a, b, c, d and f) represent significant differences ($P < 0.01$, except for a and b, where the level of significance is $P < 0.05$).

of high dose (UV-B (H)) and low dose (UV-B (L)) radiation, while in the presence of Se, effective quantum yield was lower in (UV-B (H)) treated algae.

The addition of Se to growth medium was found to enhance ETS activity in UV-B (L) treated *Zygnema* (Fig. 2). When *Zygnema* was exposed to UV-B (H) dose in the presence of either concentration of Se in the growth medium, ETS activity was observed to be lower than in algae exposed to UV-B (L) dose (Fig. 2). Addition of Se significantly decreased ETS activity in UV-B (H) irradiated algae.

Discussion

Neither UV-B irradiation nor Se, either singly or together, affected the optimal quantum yield of PSII (Fv/Fm) (Fig. 1A). According to Bischof et al. (1998) unaffected Fv/Fm is an indication that the antenna complex is not damaged irreversibly.

It is of much interest, that effective quantum yield of PSII and ETS activity in *Zygnema* showed the same pattern regarding different UV-B doses and addition of Se. Effective quantum yield of PSII was lower in the presence of Se in UV-B (H) treated algae comparing to UV-B (L) treated algae (Fig. 1B). Similarly ETS activity in algae, exposed to UV-B (H) dose, was observed to be lower than in algae exposed to UV-B (L) dose (Fig. 2). Both parameters showed that UV-B radiation negatively affected the flow of electrons in PSII and respiratory chain. Additionally, effective quantum yield of PSII and ETS activity were lower in the presence of Se in UV-B (H) treated algae (Figs. 1B and 2). Present results supported the proposal that Se added to UV-B (H) irradiated algae disturbed targets on the electron transport side of the PSII reaction center and respiratory chain, resulting in less efficient use of energy. Schofield et al. (1995) reported that the decrease in effective quantum yield reduces the flow of electrons out of PSII and lowers the rates of ATP and NADPH$_2$ formation. UV-B radiation thus interferes with the mechanisms involved in the utilization and beneficiary effect of Se.

Under low UV-B dose (UV-B (L)) radiation algae responded to elevated level of Se by both sources of energy, namely by higher photosynthetic yield and by enhanced ETS activity (Figs. 1B and 2). That indicated that Se was beneficial for the plant metabolism, although it did not mitigate UV-B stress in this particular alga. The enhancement of ETS activity by the addition of Se in UV-B (L) treated *Zygnema* (Fig. 2) is consistent with findings that supplementation of Se in the medium of *Vigna radiata* enhanced respiratory and succinate dehydrogenase activity and the involvement of Se in mitochondrial membrane functions (Easwari & Lalitha 1995). Present observations on ETS activity indicated that some of the respiratory enzymes or other molecules of mitochondria require Se for their activity. It is evidenced (Ursini et al. 1999), that Se is needed for proper function of mitochondria in animal spermatozoa. The importance of Se for activity of the mitochondria is possibly an evolutionary recollection since these organelles originate from an endosymbiotic bacterium.

The results of this investigation clearly show that Se is involved in the activation of energy resources in green alga *Zygnema*, and establish the importance of this trace element for lower plants.

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References

Bischof K., D. Hanelt & Wiencke C. 1998. UV-radiation can affect depth-zonation of Antarctic macroalgae. Mar. Biol. **131:** 597–605.

Björkman O. & Demmig-Adams B. 1995. Regulation of photosynthesis light energy capture, conversion, and dissipation in leaves of higher plants, pp. 17–47. In: Schulze E.D. & Caldwell M.M. (eds), Ecophysiology of Photosynthesis, Ecological Studies, Springer Verlag, Berlin.

Björn L.O. 1999. UV-B Effects: Receptors and Targets, pp. 821–832. In: Singhal G.S., Renger G., Sopory S.K., Irrgang K.D. & Govindjee (eds), Concepts in Photobiology: Photosynthesis and Photomorphogenesis, Narosa Publishing House, New Delhi, India.

Caldwell M.M. 1968. Solar ultraviolet radiation as an ecological factor for Alpine plants. Ecol. Monogr. **38:** 243–268.

Cullen J.J. & Neale P.J. 1994. Ultraviolet radiation, ozone depletion, and marine photosynthesis. Photosynth. Res. **39:** 303–320.

Easwari K. & Lalitha K. 1995. Subcellular-distribution of selenium during uptake and its influence on mitochondrial oxidations in germinating *Vigna radiata* L. Biol. Trace Elem. Res. **48:** 141–160.

Ekelund N.G.A. & Danilov R.A. 2001. The influence of selenium on photosynthesis and "light-enhanced dark respiration" (LEDR) in the flagellate *Euglena gracilis* after exposure to ultraviolet radiation. Aquat. Sci. **63:** 457–465.

Eker A.P.M., Kooiman P., Hessels J.K.C. & Yasui A. 1990. DNA photoreactivating enzyme from the cyanobacterium *Anacystis nidulans*. J. Biol. Chem. **265:** 8009–8015.

Fu L.H., Wang X.F., Eyal Y., She Y.M., Donald L.J. & Standing K.G. 2002. A seleno protein in the plant kingdom. J. Biol. Chem. **277:** 25983–25991.

Gaberščik A., Germ M., Škof A., Drmaž D. & Trošt T. 2002. UV-B radiation screen and respiratory potential in two aquatic primary producers: *Scenedesmus quadricauda* and *Ceratophyllum demersum*. Verh. Internat. Verein Limnol. **27:** 422–425.

Gehrke C., Johanson U., Gwinn-Jones D., Björn L.O., Callaghan T.V. & Lee J.A. 1996. Single and interactive effects of enhanced ultraviolet-B radiation and increased atmospheric CO_2 on terrestrial and subarctic ecosystems. Ecol. Bull. **45:** 192–203.

Harrison P.J., Yu P.W., Thompson P.A., Price N.M. & Philips D.J. 1988. Survey of selenium requirements in marine phytoplankton. Mar. Ecol. Prog. **47:** 89–96.

Hazzard C., Lesser M.P. & Kinzie III.R.A. 1997. Effects of ultraviolet radiation on photosynthesis in the subtropical marine diatom *Chaetoceros gracilis* (Bacilariophyceae). J. Phycol. **33:** 960–968.

Häder D.P., Lebert M. & Helbling E.W. 2001. Effects of solar radiation on the Patagonian macroalga *Enteromorpha linza* (L.) J. Agardh Chlorophyceae. J. Photochem. Photobiol. B: Biol. **62:** 43–54.

Karsten U., Bischof K., Hanelt D., Tug H. & Wiencke C. 1999. The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). Physiol. Plant. **105:** 58–66.

Kenner R.A. & Ahmed S.I. 1975. Measurements of electron transport activities in marine phytoplankton. Mar. Biol.**33:** 119–127.

Kreft I., Stibilj V. & Trkov Z. 2002. Iodine and selenium content in pumpkin (*Cucurbita pepo* L.) oil and oil-cake. Eur. Food Res. Technol. **215:** 279–281.

Krizek D.T. & R. Mirecki M. 2004. Evidence for phytotoxic effects of cellulose acetate in UV exclusion studies. Environ. Exp. Bot. **51:** 33–43.

Li Z.Y., Guo S.Y. & Lin L. 2003. Bioeffects of selenite on the growth of *Spirulina platensis* and its biotransformation. Bioresource Technol. **89:** 171–176.

Mittler R. & Tel-Or E. 1991. Oxidative stress responses in the unicellular cyanobacterium *Synechococcus* PCC7942. Free Rad. Res. Comm. **12**: 845–850.

Packard T.T. 1971. The measurement of respiratory electron-transport activity in marine phytoplankton. J. Mar. Res. **29:** 235–243.

Rozema J., van de Staaij J. & Tosserams M. 1997. Effects of UV-B radiation on plants from agro- and natural ecosystems, pp. 213–232. In: Lumsden P.J. (ed), Plants and UV-B, responses to environmental change, Cambridge University Press, Cambridge, New York, Melbourne.

Schreiber U. & Bilger W. 1992. Progress in chlorophyll fluorescence research: major developments during the last years in retrospect. Prog. Bot. **54**: 151–173.

Schreiber U., Bilger W. & Neubauer C. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis, pp. 49–69. In: Schulze E.D. & Caldwell M.M. (eds), Ecophysiology of Photosynthesis, Ecological Studies, Springer-Verlag, Berlin.

Schofield O., Kroon B.M.A. & Prézelin B.B. 1995. Impact of ultraviolet-B radiation on photosystem II activity and its relationship to the inhibition of carbon fixation rates for Antarctic ice algae communities. J. Phycol. **31:** 703–715.

Seppänen M., Turakainen M. & Hartikainen H. 2003. Selenium effects on oxidative stress in potato. Plant Sci. **165:** 311–319.

Töth G.L. 1993. Electron transport system (ETS) activity of the plankton, sediment and biofilm in Lake Balaton (Hungary). Verh. Internat. Verein Limnol. **25**: 680–681.

Ursini F., Heim S., Kiess M., Maiorino M., Roveri A., Wissing J. & L. Flohe 1999. Dual function of the selenoprotein PHGPx during sperm maturation. Science **285:** 1393–1396.

Yao Y., Li Y., Yang Y. & C. Li 2005. Effect of seed pretreatment by magnetic field on the sensitivity of cucumber (*Cucumis sativus*) seedlings to ultraviolet-B radiation. Environ. Exp. Bot. **54:** 86–294.

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