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Effect of gamma irradiation on physiological and proteomic changes of Arctic *Zygnema* sp. (Chlorophyta, Zygnematales)

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Abstract: In this study, the effect of ionizing radiation on an Arctic alga was examined by assessing photosynthetic efficiency, antioxidant capacity, and proteomic changes. Arctic *Zygnema* sp. was gamma-irradiated at doses of 1, 3, and 5 kGy and showed serious cell damage after irradiation with the 5 kGy dose. The photosynthetic efficiency markedly decreased in gamma-irradiated *Zygnema* sp.; whereas, antioxidant capacity significantly increased. To investigate changes in protein expression levels, two-dimensional electrophoresis was performed. Thirty-nine protein spots were differently expressed. Several proteins, both upregulated and downregulated, were identified as photosynthesis-related proteins, confirming an important effect of gamma irradiation on the photosynthetic process. On the one hand, proteins similar to those involved in energy metabolism, isoprene biosynthesis, and protein biosynthesis were significantly downregulated. On the other hand, proteins related to DNA repair, quinone oxireductase, regulation of microtubules, and cell wall biogenesis were upregulated in gamma-irradiated *Zygnema* sp. These results provide insights on the effects of gamma irradiation on *Zygnema* sp. that can aid the interpretation of response mechanisms of Arctic algae to ionizing radiation.

Key Words: 2D-electrophoresis, Antioxidant, Arctic *Zygnema* sp., Gamma irradiation

INTRODUCTION

In Arctic regions, low temperatures, lack of nutrients, and extreme seasonal variations of incoming cosmic rays are physical and chemical environmental stressors that limit the biodiversity of Arctic organisms. However, some freshwater and terrestrial algae have successfully adapted to the Arctic environment and can grow under these extreme environmental conditions (Kim *et al*. 2008). Cosmic rays are a primary source of radiation in Arctic regions and a particularly strong environmental stressor of Arctic biological systems because of high latitudes and thinning of the ozone layers. Ionizing irradiation has been a major stress in an evolutionary context; in today's atmosphere it can be ruled out, as all electromagnetic radiation with energies > 10 eV are effectively blocked. But, it can be expected that it will increase due to human activities affecting the ozone layer. Recent reports on the depletion of the ozone layer and subsequent effects of cosmic rays are more concerning than ever (Lu & Sanche 2001).

Recently, many studies have focused on the effects of ultraviolet (UV) radiation caused by the depletion of the ozone layer on plants. These studies described alterations in photosynthesis and growth (Ruhland *et al*. 2005; Germ *et al*. 2005; Tao *et al*. 2010), most possibly due to oxidative stress (Yannarelli *et al*. 2006) and DNA damage (Bray & West 2005). However, UV radiation is non-ionizing and therefore a different effect of ionizing radiation from cosmic rays on biological systems could be expected.

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Ionizing radiation is known to disrupt the permeability and organization of cell membranes mostly via free radical–induced lipid peroxidation and protein modifications (Leyko & Bartosz 2000). For example, exposure of plant cells to 5 kGy ionizing radiation was shown to result in a complete breakdown of the cell wall middle lamella (Kovács *et al*. 1995; Kovács & Keresztes 2002). With respect to metabolism, ionizing radiation alters photosynthesis, modulates antioxidant systems, changes malondialdehyde (a marker of oxygen free radicals) levels, and enhances the production of phenolic compounds (Kim *et al*. 2004; Wi *et al*. 2005). Furthermore, through the production of reactive oxygen species (ROS), ionizing radiation can induce cellular antioxidant defence enzymes, such as superoxide dismutase, glutathione peroxidase, and the thioredoxin system (Mittler *et al*. 2004).

Although algae play an important ecological role and are considered strong indicators of environmental changes, few studies have investigated their responses to ionizing radiation (De Micco *et al*. 2011; Singh *et al*. 2013; Yoon *et al*., 2013a, 2013b; Choi *et al*., 2014; Mavi *et al*. 2014). To our knowledge, there is no previous report on the response of an Arctic alga to ionizing radiation.

The purpose of this study was to examine the effect of ionizing radiation on an Arctic *Zygnema* sp. *Zygnema* is often found in shallow puddles and streamlets and on the surface of water-saturated soil and stone particles exposed to ambient solar radiation (Herburger *et al*. 2015). Several studies have investigated the susceptibility or tolerance of *Zygnema* sp. to UV radiation (Germ *et al*. 2009; Holzinger *et al*. 2009; Pichrtová *et al*. 2013). The studies by Pichrtová *et al*. (2014a, 2014b) in particular illustrate phylogenetic relationships of the strains of *Zygnema* and their desiccation tolerance. Therefore,

we considered *Zygnema* sp. a good model organism to examine the effect of ionizing radiation on Arctic algae. To this end, we compared photosynthetic efficiency and antioxidant capacity of gamma-irradiated Arctic *Zygnema* sp. to those of non-irradiated controls. Additionally, we performed two-dimensional polyacrylamide gel electrophoresis (2-DE) to compare protein profiles between gamma-irradiated and non-irradiated *Zygnema* sp. The functional implications of differentially expressed proteins in gamma-irradiated Arctic *Zygnema* sp. are discussed.

MATERIAL AND METHODS

Arctic *Zygnema* sp. collected from Ny-Ålesund (78°56′N, 11°56′E) was obtained from Kongju National University (Kongju, South Korea), and was cultured in Bold's basal medium (Bischoff and Bold 1963) at 4°C with continuous cool-white fluorescent lighting (> 20 μmol photons·m^{-2}·s^{-1}).

Arctic *Zygnema* sp. was transferred into 50 mL tubes and irradiated with increasing doses of 1, 3, and 5 kGy with a ^{60}Co irradiator (cylinder shape with ^{60}Cobalt pencils, AECL, IR-79, Nordion, Ottawa, Canada) at a dose rate of 10 kGy h^{-1}. The amount of *Zygnema* sp. in the tube was about 0.01 mg wet weight. For a dose of 1 kGy, the cells were incubated for 6 min under exposure. This experiment was performed at Advanced Radiation Technology Institute, Korean Atomic Energy Research Institute (Jeongeup, South Korea). Non-irradiated *Zygnema* sp. was used as control. After gamma irradiation, samples were incubated in the same culture conditions as above (see Strain and culture conditions) for 2 h before further analyses.

To assess the sensitivity of Arctic *Zygnema* sp. to gamma irradiation, gamma-irradiated cells were stained using fluorescein diacetate (FDA; Steward *et al*. 1999) and illuminated by blue light in a fluorescence microscope (Scope A1, Zeiss, Oberkochen, Germany). Cells emitting green fluorescence were considered viable.

Chlorophyll fluorescence was measured with an Imaging-Pam Chl fluorometer (Walz, Effeltrich, Germany) following manufacturer's instructions. Samples were dark-acclimated for 10 min at room temperature prior to the readings. The *Fv*/*Fm* ratio represents the maximal photochemical efficiency of Photosystem II (PS II; Krause & Weis 1991). Photochemical quenching (qP) was measured by analyzing chlorophyll fluorescence quenching with the same fluorometer.

To prepare ethanol extracts of *Zygnema* sp. to be used in further analyses, samples (500 mg) of gamma-irradiated and non-irradiated algal material were ground in liquid nitrogen, homogenized in 10 mL of ethanol, and stirred at 4°C. After 48 h, the homogenates were centrifuged (20,000 × *g* for 20 min at 4°C) to remove residues. Supernatants were filtered through a 0.45 μm filter (Millipore Corporation, Billerica, Massachusetts USA) and concentrated in rotary evaporator (Eyela, model No. N-1001s-W, Tokyo, Japan). The resulting ethanol extracts were weighed and stored at 4°C.

We used a free radical, 2-diphenyl-1-picrylhydrazyl (DPPH), to assess the free radical scavenging (antioxidant) activity of various samples. The DPPH radical scavenging activity of gamma-irradiated and non-irradiated extracts of *Zygnema* sp. (1 mg/mL) was evaluated using the method developed by Okada & Okada (1998).

The ferric reducing ability of plasma (FRAP) assay measures the ability of a compound to reduce a ferric-tripyridyltriazine complex (Fe^{+}-TPTZ) to the ferrous form (Fe^{2+}) by electron transfer and hence indicates the antioxidant power of the compound. FRAP assay of gamma-irradiated and non-irradiated ethanol extracts of *Zygnema* sp. (3 mg mL^{-1}) was performed using the method developed by Benzie & Strain (1996).

The total phenolics content (TPC) of a sample is another indicator of its oxidant capacity. Gamma-irradiated and non-irradiated ethanol extracts of *Zygnema* sp. (0.9 mL) at a concentration of 3 mg/mL were mixed with 0.1 mL of ascorbic oxidase (50 units mL^{-1}) followed by incubation at 23°C for 90 min to remove the L-ascorbic acid. The L-ascorbic acid–free extracts (0.1 mL) were then mixed with 0.2 mL of Folin–Ciocalteu reagent (Sigma Chemical Co., St. Louis, Missouri USA) and incubated at 23°C for 1 min. Following incubation, 3 mL of 5% Na_2CO_3 was added and the samples were further incubated at 23°C for 2 h. Absorbance of samples was then measured at 765 nm using a spectrophotometer. The TPC of ethanol extracts (in μg mL^{-1}) was calculated from a standard curve prepared with gallic acid.

We performed 2-DE according to modified version of the method developed by Phee & Bhoo (2003). After 2-DE, the gel was stained with Coomassie brilliant blue for 1 h and treated with a de-staining solution of 10% (v/v) methanol and 10% (v/v) acetic acid.

Gels were scanned with a photo scanner (Perfection V700 Photo, Epson Corp., Nagano, Japan) and images analyzed using PDQuest software (Bio-Rad 2015). Differentially expressed protein spots were selected to compare gamma-irradiated (3 kGy) to non-irradiated samples.

Differentially expressed protein spots were separated from the gel after image analysis and digested according to the method of Koksharova *et al*. (2007). Protein identification was performed using a 4700 Proteomics Analyzer (Applied Biosystems, Foster City, California USA). Peptides were ionized with a Nd:YAG 200 Hz laser at 335 nm, and mass profiles were calibrated using MoverZ software (MoverZ 2015). Peptide masses were compared with protein databases (NCBInr and Swiss-Prot) using the Mascot software (Matrix Science 2015).

Means and standard deviations were calculated using the Statistical Package for Social Sciences software (SPSS 2000). Student's two-tailed *t*-tests with significance levels of $*p < 0.05$ and $**p < 0.01$ were used to assess differences between two sample means.

RESULTS

Sensitivity of Arctic *Zygnema* sp. to gamma radiation

To investigate the effect of ionizing radiation, Arctic *Zygnema* sp. was gamma-irradiated at increasing dosed of 1, 3, and 5 kGy, and cell viability was assessed with FDA staining. Algal cells gamma-irradiated at doses of 1 and 3 kGy were mostly viable and comparable to the non-irradiated control (0 kGy),

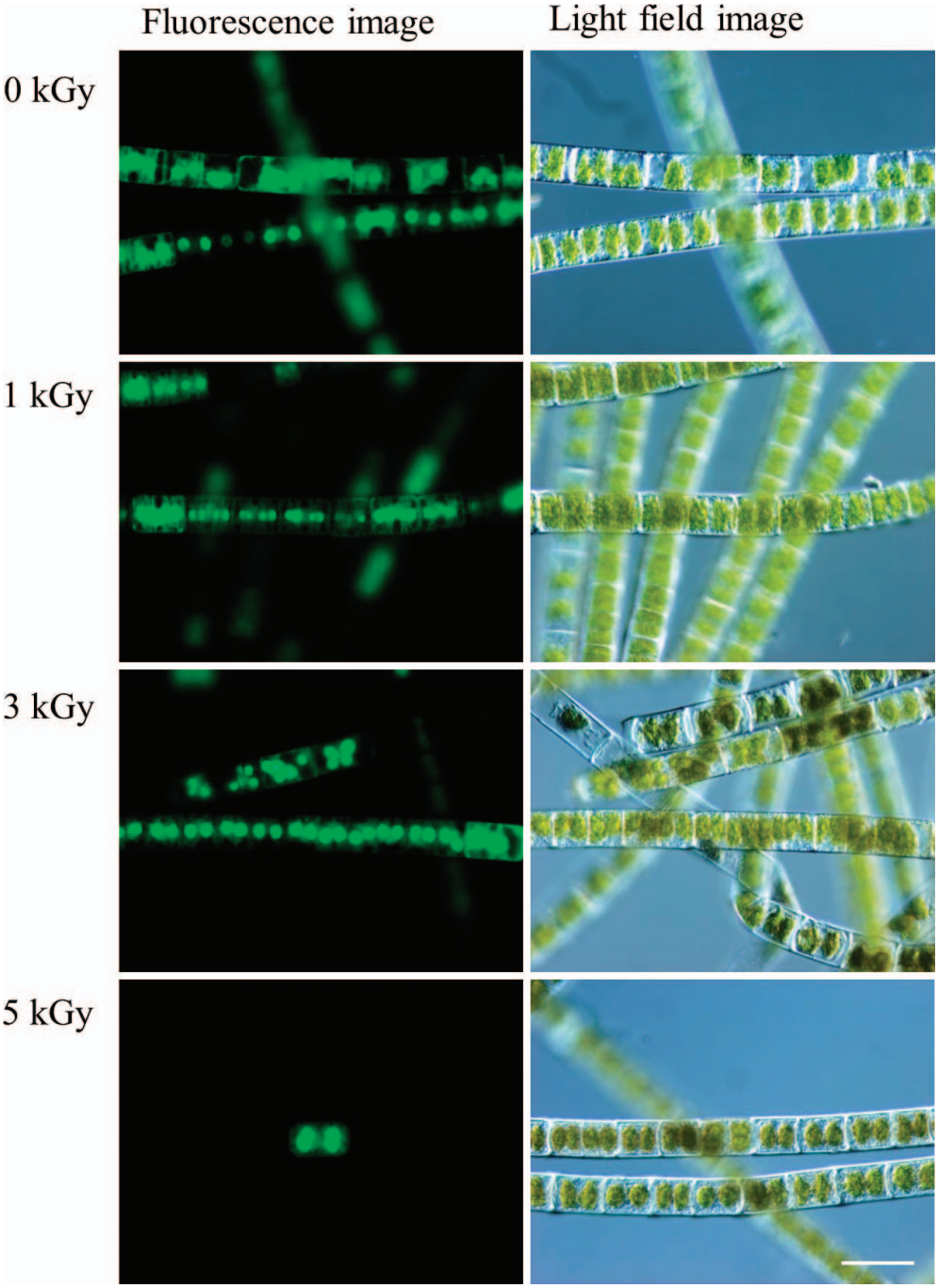


Fig. 1. Evaluation of cell viability of *Zygnema* sp. after exposure to increasing doses of gamma irradiation. Cell viability was assessed by fluorescein diacetate (FDA) staining. Scale bar = 50 μm.

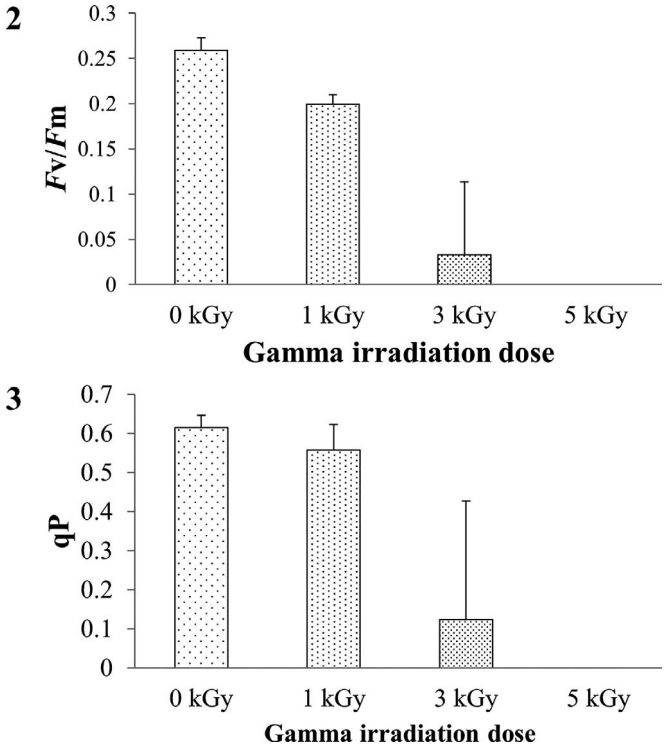
while FDA staining was barely detected in cells irradiated with a dose of 5 kGy (Fig. 1). This result suggested that 5 kGy of gamma irradiation is an acute lethal dose to Arctic cells of *Zygnema* sp.

Effect of gamma irradiation on chlorophyll fluorescence parameters

Maximal photochemical efficiency (*Fv*/*Fm*) and qP were measured to determine the effect of gamma irradiation on the photosynthetic activity of Arctic *Zygnema* sp. Compared with non-irradiated control sample (0 kGy), all irradiated groups exhibited significant decreases in their *Fv*/*Fm* and qP values (Figs 2 and 3, respectively) that depended on the irradiation dose.

Effect of gamma irradiation on antioxidant capacity

We investigated the effect of gamma irradiation on the antioxidant capacity of Arctic *Zygnema* sp. using three



Figs 2–3. Effect of gamma irradiation on chlorophyll fluorescence parameters. Values are expressed as means ± SD ($n = 7$). Differences in the mean values between gamma-irradiated samples and non-irradiated controls (0 kGy) were examined using Student's two-tailed *t*-test with significance levels of $*p < 0.05$ and $**p < 0.01$.
Fig. 2. Maximal PS II photochemical efficiency (*Fv*/*Fm*).
Fig. 3. Photochemical quenching (qP).

different methods: determination of DPPH radical scavenging activity, FRAP assay, and determination of TPC. The DPPH radical scavenging activities of gamma-irradiated and non-irradiated control samples (0 kGy) are shown in Fig. 4. Radical scavenging activity significantly increased in the gamma-irradiated samples compared with non-irradiated controls. The highest radical scavenging activities were observed at radiation doses of 1 and 3 kGy.

The reducing power measured by FRAP assay and the TPC were similarly altered by gamma irradiation (Figs 5 and 6, respectively). FRAP and TPC values significantly increased in the gamma-irradiated samples compared with non-irradiated controls, with highest values being achieved at irradiation doses of 1 and 3 kGy.

Altogether, results from the three methods indicate that gamma irradiation increased the antioxidant capacity of *Zygnema* sp.

Differentially expressed proteins in gamma-irradiated *Zygnema* sp.

To identify differentially expressed proteins between gamma-irradiated and non-irradiated *Zygnema* sp., protein expression levels were analyzed by 2-DE. The protein profiles of non-irradiated and gamma-irradiated *Zygnema* sp. are shown in Figs 7 and 8, respectively. Thirty-nine protein spots were altered by gamma irradiation. Among the differentially expressed proteins, 20 proteins were downregulated (indicated in Fig. 7), and 19 proteins were upregulated (indicated in Fig. 8) by gamma irradiation. Of the 20 downregulated proteins, 12 significantly matched known proteins in protein databases (Table 1). These were related to photosynthesis (Spots No. 1, 5, 7, 9, 12, 13, 16, and 17), energy metabolism (Spot No. 8), isoprene biosynthesis (Spots No. 2 and 4), and protein biosynthesis (Spot No. 10). The Mascot score and sequence coverage ranged from 30%–80% and 5%–97%, respectively.

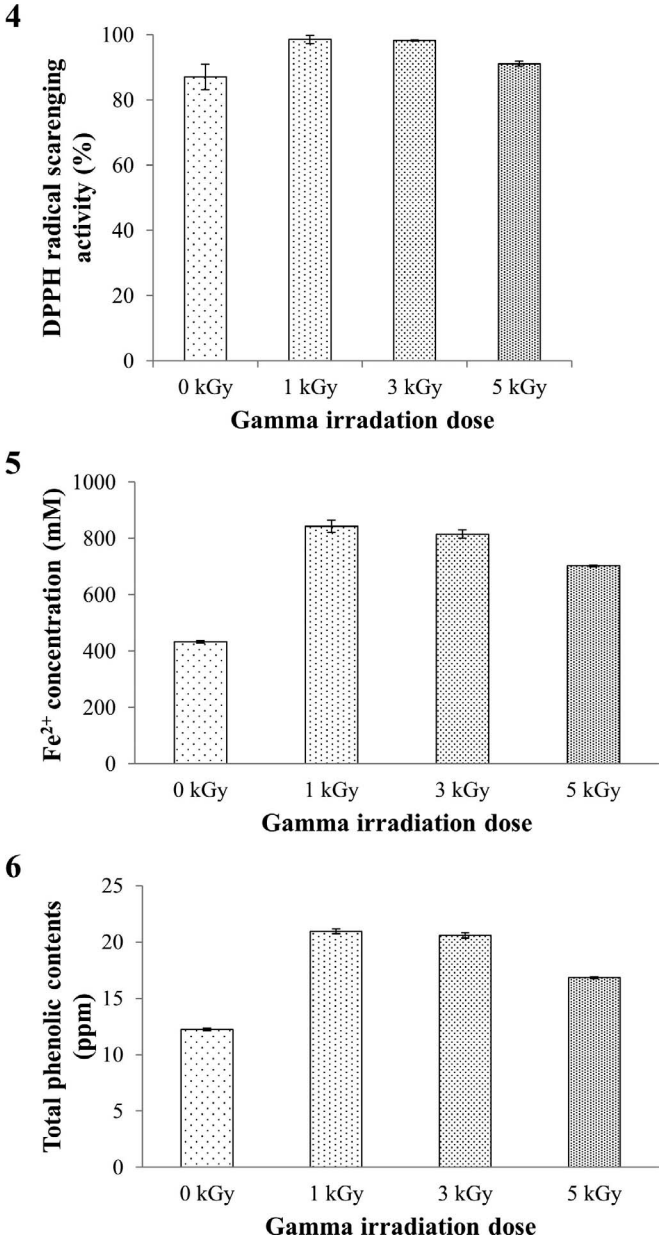
Of the 19 upregulated proteins, eight were similarly identified, with Mascot scores and sequence coverage ranging from 43% to 127% and from 17% to 34%, respectively (Table 2). These upregulated proteins were related to quinone oxidoreductase (Spot No. 24), plant cell wall biogenesis (Spot No. 25), microtubule-based movement (Spot No. 26), DNA repair (Spots No. 34 and 35), and photosynthesis (Spots No. 33, 37, and 38).

DISCUSSION

Understanding the adaptations of Arctic species to ionizing radiation is an important component of understanding survival in the extreme environmental conditions of the Arctic. In particular, a significant depletion of the Arctic ozone layer is bound to have dramatic repercussion on this region's biological systems. In this study, the effect of ionizing radiation on Arctic algae was studied by examining photosynthetic efficiency, antioxidant capacity, and proteomic changes of gamma-irradiated *Zygnema* sp.

Per the definition of the grey (Gy) unit, it is the absorption of one Joule of radiation energy by 1 kg of matter. Therefore, the amount of algae present in the tube is important. Gamma radiation can cause radiolysis of water (leading to ROS). This might drastically influence the effect on organic compounds like nucleotides. Also, in the filamentous *Zygnema* sp., the cell responses might not be homogeneous. After gamma radiation of conjugating green algae *Sirogonium* sp., the formation of giant cells was promoted by the treatment and observed over a long time period of up to 6 mo. Nuclear size was significantly increased, which could be beneficial for surviving extreme conditions (Wells & Hoshaw 1980).

The *Fv*/*Fm* value in this study was low, at 0.25. In the previous study, Pichrtová *et al*. (2013) reported *Fv*/*Fm* values between 0.4 and 0.7 for Arctic and Antarctic strains of *Zygnema*. However, it does not surprise us that gamma irradiation has a drastic impact on the status of PS II. Photosynthesis may be the most thoroughly studied process in plant biology. Owing to its central role in plant metabolism and significance to all oxygen-dependent life on Earth, studies on the adverse effects of global environmental changes on photosynthesis are of particular interest. The common effects of radiation on photosynthetic function include decreased CO_2 fixation and oxygen evolution (Allen *et al*. 1997), which could be caused by several molecular events. Most studies have found that PS I is only minimally affected by radiation (Iwanzik *et al*. 1983; Renger *et al*. 1989); whereas, PS II appears to be a more important target (Bornman 1989). It is likely that radiation causes an



Figs 4–6. Effect of gamma irradiation on antioxidant capacity. Values are expressed as means ± SD ($n = 7$). Differences in the mean values between gamma-irradiated samples and non-irradiated controls (0 kGy) were examined using Student's two-tailed *t*-test with significance levels of $*p < 0.05$ and $**p < 0.01$.

Fig. 4. 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (%).

Fig. 5. Fe^{2+} concentration (mM) measured by ferric reducing ability of plasma (FRAP) assay.

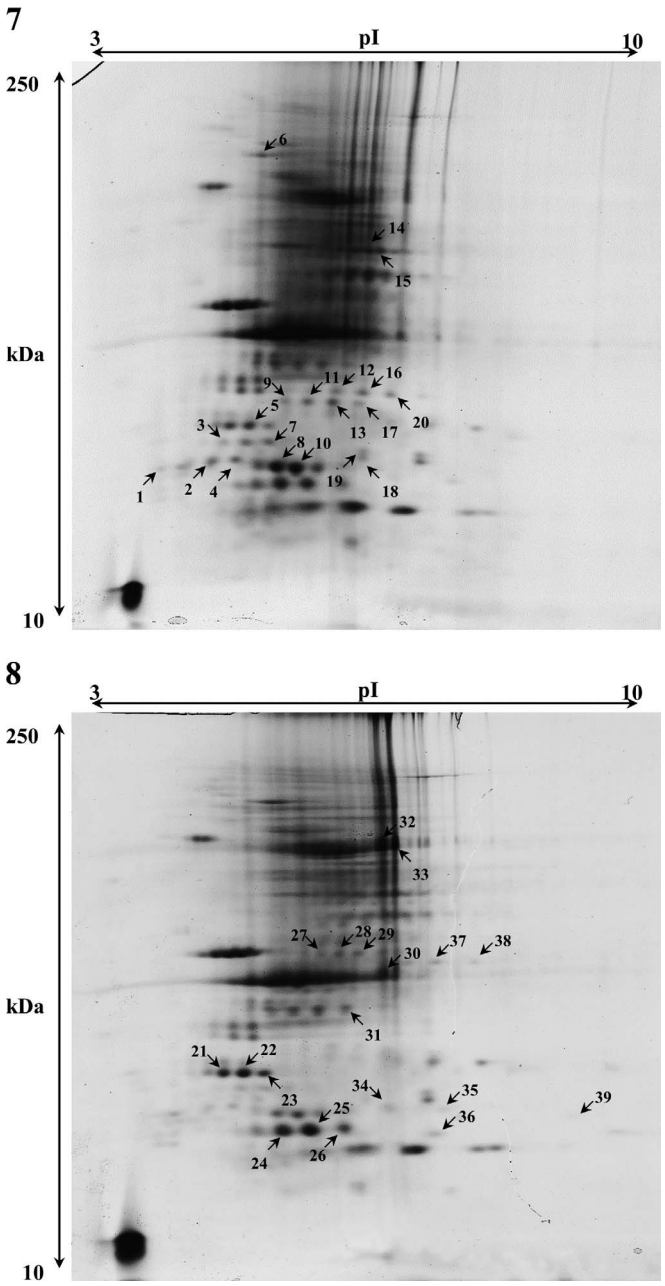
Fig. 6. Total phenolic content (μg/mL).

inhibition of energy transfer within the PS II reaction centre by blocking electron flow. In this study, maximal photochemical efficiency (*Fv*/*Fm*) and qP were remarkably decreased in gamma-irradiated *Zygnema* sp., suggesting that the photosynthetic efficiency of this alga may be negatively affected by ionizing radiation.

It has been proposed that cellular responses to radiation occur via direct antioxidant effects on ROS produced by water radiolysis. Antioxidant activity has been attributed to various mechanisms, including prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reductive capacity, and radical scavenging (Mallick & Mohn, 2000). The radical scavenging activity and reductive capacity can be significant indicators of potential antioxidant activity (Benzie & Strain, 1996). In this study, the increase of the DPPH radical scavenging activity and reductive capacity in gamma-irradiated *Zygnema* sp. indicates an enhanced antioxidant capacity and thus higher tolerance to oxidative stress caused by radiation. In plants, the antioxidant system is significantly affected by ionizing radiation. For instance, a recent study reported that plant leaves exposed to ionizing radiation exhibited an increase in flavonoid and polyphenol content (Santos *et al*. 2004). Plants have evolved several mechanisms to cope with photochemical damage, one of the most important of which involves shielding from ionizing radiation through flavonoid accumulation in the leaf epidermis (Robberecht & Caldwell 1983; Schmelzer *et al*. 1988). Polyphenolic compounds like flavonoids exert an antioxidant activity due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Kim *et al*. 2009). Here, Arctic *Zygnema* sp. exposed to gamma irradiation also exhibited increased total phenolic content. Therefore, the high phenolic content induced by gamma irradiation likely plays an important role in reducing the negative effects of oxidative stress for algal cell survival. In Zygnematophyceae, phenolics play an important role in protection and avoidance of antioxidative stress. Aigner *et al*. (2013) demonstrated an increase in phenolics in sun exposed purple filaments of *Zygogonium* using the Folin–Ciocalteu assay. The concentration of total phenolics was reported about 30 mg g^{-1} dry weight gallic acid equivalents in the previous study but the concentration from our non-irradiation *Zygnema* sp. was about 4.1 mg g^{-1}.

Proteomic analysis using 2-DE is a sensitive and powerful technique. Combined with mass spectrometry (MS), it allows rapid and reliable protein identification by peptide fingerprinting. In recent years, proteomic-based technologies have been successfully applied to the systematic study of proteomic responses to a wide range of abiotic stresses, including temperature (Sule *et al*. 2004; Yan *et al*. 2006), oxidative stress (Wang *et al*. 2004), and UV radiation (Casati *et al*. 2005). Kim *et al*. (2007) studied the effect of gamma irradiation on *Arabidopsis* leaves by transcriptomic profiling. In that study, defence/stress response genes were upregulated but rhythm/growth responses were downregulated.

In our proteomic analyses we identified 20 proteins among 39 that were altered by gamma irradiation (Tables 1, 2). Most of the identified proteins downregulated by gamma irradiation were related to photosynthesis (Spots No 1, 5, 7, 9, 12, 13, 16, and 17). Among these, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) was the most common protein. RuBisCO is associated with carbohydrate metabolism and is the key enzyme for CO_2 assimilation in the Calvin cycle. It also catalyzes the oxygenation of ribulose-1,5-bisphosphate (RuBP), in which oxygen competes with CO_2 as a substrate at the same active site (Hartman & Harpel 1994). Interestingly, although most (six)



Figs 7–8. Protein profiles of *Zygnema* sp. by two-dimensional electrophoresis.

Fig. 7. Protein profiles of non-irradiated *Zygnema* sp. Numbers and arrows indicate spots of downregulated proteins on two-dimensional electrophoresis gel.

Fig. 8. Protein profiles of gamma-irradiated (3 kGy) *Zygnema* sp. Numbers and arrows indicate spots of upregulated proteins on two-dimensional electrophoresis gel.

RuBisCo protein spots were downregulated in our study, three spots seemed to be upregulated. This is similar to results of Casati *et al*. (2005), where RuBisCO large subunit expression was either upregulated or downregulated in response to UV radiation.

Another protein downregulated in gamma-irradiated *Zygnema* sp. was identified as adenosine triphosphate (ATP) synthase (Spot No. 8). A central role of ATP synthase is to convert the transthylakoid proton gradient into ATP and is related to cell growth. Two downregulated protein spots (Spots No. 2 and 4) had high similarity to acetolactate synthase and 1-deoxy-D-xyluloase 5-phosphate reductoisomerase, respectively. These proteins are involved in isoprene biosynthesis and previously reported to be downregulated in *Haematococcus pluvialis* Flotow by oxidative stress (Wang *et al*. 2004).

Finally, one of the identified proteins downregulated in gamma-irradiated *Zygnema* sp. was related to protein biosynthesis (Spot No. 10). Elongation factor Tu is an essential component of the protein synthesis pathway and plays a role in polypeptide elongation. It interacts with aminoacyl-tRNA and transports the codon-specific tRNA to the aminoacyl site on the ribosome (ribosomal A site) during the translation elongation step (Baldauf & Palmer 1990).

Brosché & Strid (2003) reviewed literature on the alteration of gene expression by ionizing radiation, including genes associated with DNA repair. Among the upregulated proteins in gamma-irradiated *Zygnema* sp., DNA ligase (Spot No. 34) and ATP-dependent DNA helicase (Spot No. 35), both related to DNA repair, were identified. In particular, DNA ligase is induced by gamma irradiation and required to repair single-strand DNA breaks caused by gamma irradiation and alkylating reagents (Caldecott *et al*. 1994; West *et al*. 2000). Therefore, DNA repair-related proteins may be upregulated in Arctic *Zygnema* sp. for radioresistance against oxidative stress caused by ionizing radiation.

In our proteomic results, another two proteins, alpha-trehalose-phosphate synthase and armadillo repeat-containing kinesin-like protein, were specifically expressed after exposure to gamma irradiation. Trehalose (Spot No. 25) is a nonreducing disaccharide composed of two glucose units. It was believed that trehalose was present only in desiccation-tolerant plants, such as *Selaginella lepidophylla* (Hook. & Grev.) Spring and *Myrothamnus flabellifolius* Welw. (Adams *et al*. 1990; Müller *et al*. 1995); however, it can be synthesized by diverse organisms, such as bacteria, fungi, lichens, algae, and invertebrates (Augier 1954; Goddijn & van Dun 1999; Elbein *et al*. 2003). In plants, trehalose is associated with cell wall biogenesis (Gómez *et al*. 2006), and its accumulation confers high tolerance to abiotic stress (Garg *et al*. 2002). The armadillo repeat-containing kinesin-like protein (Spot No. 26) regulates microtubules during biological processes and is involved in the ATP-binding mechanism. The interaction of kinesin with mitochondria might also influence the nuclear positioning (Holzinger & Lutz-Meindl 2002). However, the specific expression patterns and functions of these two proteins following exposure to gamma irradiation are not clear in algae and need to be investigated further.

The last identified upregulated protein was the ubiquinone oxidoreductase 13-kD–like subunit (Spot No. 24). This protein was related to the mitochondrial nicotinamide adenine dinucleotide protein (Popescu *et al*. 2006) and was overexpressed in *Nannochloropsis oculata* (Droop) Hibberd by oxidative stress produced by cadmium (Kim *et al*. 2005).

In conclusion, we demonstrate in this study that gamma irradiation can reduce photosynthetic efficiency, increase antioxidant capacity, and change protein expression in Arctic *Zygnema* sp. Our results suggest that photoinhibition

Table 1. Summary of downregulated proteins identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS).

Spot No.	Regulation by γ radiation	Protein name	Species	Accession No.	Mr/pI[1]	Mascot score (%)	SC (%)[2]	Expression change (ratio)
Photosynthesis								
1	down	light-independent protochlorophyllide reductase subunit N	*Porphyra yezoensis* Ueda	CHLN_PORYE	49660/7.48	34	15	0.35
5	down	photosystem I reaction center subunit IX	*Odontella sinensis* (Greville) Grunow	PSAJ_ODOSI	4829/4.55	31	97	0.47
7	down	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	59	20	0.44
9	down	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	64	18	0.14
12	down	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	65	15	0.49
13	down	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	80	18	0.2
16	down	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	71	17	0.12
17	down	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	68	12	0.46
Energy metabolism								
8	down	ATP synthase subunit beta, chloroplastic	*Lemna minor* L.	ATPB_LEMMI	53580/5.01	61	27	0.35
Isoprene biosynthesis								
2	down	acetolactate synthase large subunit	*Porphyra yezoensis* Ueda	ILVB_PORPU	64888/8.23	30	5	0.2
4	down	1-deoxy-D-xylulose 5-phosphate reductoisomerase, chloroplastic	*Mentha piperita* L.	DXR_MENPI	51001/5.88	42	8	0.26
Protein biosynthesis								
10	down	elongation factor Tu, chloroplastic	*Pisum sativum* L.	EFTU_PEA	53017/6.62	58	20	0.47

[1] Mr/pI, nominal mass and calculated isoelectric point.
[2] SC, sequence coverage of the identified peptide sequences.

Table 2. Summary of upregulated proteins identified by MALDI-TOF MS.

Spot No.	Regulation by γ radiation	Protein name	Species	Accession No.	Mol. Mass (Mr)/pI[1]	Mascot score	SC (%)[2]	Expression change (ratio)
Quinone oxidoreductase								
24	up	NAD(P)H-quinone oxidoreductase subunit H, chloroplastic	*Vitis vinifera* L.	NDHH_VITVI	45340/5.20	46	25	2.07
Cell wall biogenesis in plants								
25	up	alpha,alpha-trehalose-phosphate synthase [UDP-forming] 6	*Arabidopsis thaliana* (L.) Heynh	TPS6_ARATH	97641/5.90	60	22	2.19
Regulation of microtubules								
26	up	armadillo repeat-containing kinesin-like protein 1	*Oryza sativa*	ARK1_ORYSJ	104685/6.58	64	18	2.72
DNA repair								
34	up	DNA ligase	*Anaeromyxobacter* sp.	DNLJ_ANASK	76283/6.33	74	17	41.53
35	up	ATP-dependent DNA helicase 2 subunit KU80	*Oryza sativa*	KU80_ORYSJ	77394/7.53	43	26	4.29
Photosynthesis								
33	up	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	100	34	3.34
37	up	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	127	29	7.4
38	up	ribulose bisphosphate carboxylase large chain	*Zygnema circumcarinatum* Czurda	RBL_ZYGCR	52690/5.83	111	21	9.47

[1] Mr/pI; Nominal mass and calculated isoelectric point.
[2] SC; Sequence coverage of the identified peptide sequences.

is induced by gamma radiation of *Zygnema* sp. and that an increased antioxidant capacity likely plays an important role in reducing the negative effects of radiation-induced oxidative stress. Proteomic analysis confirmed that photosynthetic function of *Zygnema* sp. was significantly affected by gamma irradiation. Differential protein expression also indicated that protein biosynthesis and energy metabolism are other processes that may be affected during oxidative stress caused by radiolysis. Moreover, the screening for radiation-regulated proteins confirmed the overexpression of DNA repair-related proteins in gamma-irradiated Arctic *Zygnema* sp. Overall, this study provides fundamental insights into gamma irradiation–induced proteomic and metabolic changes in an Arctic alga.

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