Differential response of growth, photosynthesis, antioxidant enzymes and lipid peroxidation to UV-B radiation in three cyanobacteria

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Abstract

Our studies indicate differential response of growth, photosynthesis, antioxidant enzymes and lipid peroxidation of three cyanobacteria: *Nostoc muscorum*, *Plectonema boryanum* and *Aphanothece* sp. to UV-B stress and the sensitivity to UV-B was maximum in *N. muscorum* and minimum in *Aphanothece* sp. Cyanobacteria exhibited varied sensitivity to UV-B radiation (280–315 nm: 0.4 W m⁻²) as 30 min of UV-B exposure caused 32, 88 and 95% growth yield after 10th day of treatment in *N. muscorum*, *P. boryanum* and *Aphanothece* sp., respectively. Photosynthetic pigment contents, whole cell oxygen yield,¹⁴C-fixation and PS II activity decreased with increasing doses of UV-B exposure (15 and 60 min), however, the inhibitory effect in *N. muscorum* was more pronounced than in *P. boryanum* and a least effect was noticed in *Aphanothece* sp. Among the photosynthetic pigments, phycocyanin was severely affected by UV-B in *N. muscorum* followed by *P. boryanum* and *Aphanothece* sp. ¹⁴C-fixation was found to be more sensitive parameter to UV-B than whole cell photosynthetic oxygen evolution. UV-B exposed spheroplasts exhibited severe damage on PS II activity and little effect on the activity of PS I. Partial restoration of PS II activity by electron donors (NH₂OH, MnCl₂ and DPC) suggested that UV-B interrupted the electron flow by affecting the component of water oxidation site as well as reaction center of PS II and the extent of damage on PS II was dependent on UV-B exposure time as well as the cyanobacterial strains. Suppression in emission peak (λmax 651 nm) of phycocyanin revealed that UV-B altered the energy transfer efficiency of phycocyanin to PS II reaction center. Furthermore, UV-B with increasing doses enhanced lipid peroxidation and the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). However, the enhancement in SOD activity in *N. muscorum* (13–25%), *P. boryanum* (18–34%) and *Aphanothece* sp. (24–53%) was high as compared with CAT and POD activity.

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1. Introduction

Anthropogenically released atmospheric pollutants such as chlorofluorocarbons (CFCs), chlorocarbons (CCs) and organo-bromides (OBs) resulted in a global reduction of the stratospheric ozone layer leading to an increased level of solar UV-B (280–315 nm) radiation at the surface of earth (Pyle, 1996; Caldwell et al., 2003; Callaghan et al., 2004). Enhanced UV-B radiation produces deleterious effects on physiological and morphological traits of plants and thus, posing a severe threat to the existence and survival of organisms (Frohnmeyer and Staiger, 2003; Prasad et al., 2005). The susceptibility to elevated UV-B irradiation is dictated by a complex interplay between protection, repair and other factors that may lead to highly variable UV-B susceptibility among the species. Some plant species are tolerant or even show stimulation, when exposed to UV-B radiation, while some are highly susceptible (Lesser et al., 1994; Xiong et al., 1995, 1996). The key factor for UV-B tolerance may be considered as UV-B absorbing pigments like scytonemin, mycosporine like amino acid (MAA), regulation of active oxygen species levels and activity of antioxidants, and the effective repair mechanism for PS II, one of the important components of photosynthetic electron transport chain (Xiong et al., 1995; Adhikary and Sahu, 1998). Cyanobacteria exhibited an extraordinary resistance to many environmental factors such as heat, cold, drought, light extremes and salinity (Foy, 1992; Tandeau de Marsac and Houmard, 1993). Since cyanobacteria were among the early photosynthetic
prokaryotes, thus it seems probable that during their evolutionary history, they might have faced more intense ambient solar UV-B radiation than others and may have acquired the ability to accumulate UV-A/UV-B screening pigments to attenuate UV-B induced damage (Sinha and Häder, 2008). In addition to this, cyanobacteria grow in diverse habitats ranging from hot springs to the Arctic and, therefore, are expected to face different levels of UV-B (Castenholz, 1997). Cyanobacteria have the ability to fix molecular nitrogen into utilizable form of nitrogen i.e. ammonia and therefore, they form a prominent component of N2-fixing microbial population in the tropical rice fields (Venkataraman, 1981; Sinha and Häder, 1996). Besides this, cyanobacteria are the greatest contributors of carbon pool in the rice soil which may enhance the microbial activity, thereby increasing the fertility of soil.

UV-B radiation impairs the photosynthesis by enhancing the rate of degradation of D1 protein of photosystem II and Rubisco enzyme and thus, declines the growth considerably (Greenberg et al., 1989; Renger et al., 1989; Trebst and Depka, 1990; Döhler and Haas, 1995; Jansen et al., 1996; Guan and Gao, 2008). Studies on higher plants suggested that the oxidizing side of PS II may be the primary target of UV-B (Bornman, 1989). However, altered Rubisco also plays a central role in the UV-B induced impairment of photosynthesis. UV-B has been shown to reduce the activity as well as polypeptide subunits of Rubisco (Jordan et al., 1992). It has been demonstrated that UV-B radiation stimulates the formation of active oxygen species (AOS) at various sites of respiratory and photosynthetic electron transport as well as during various biochemical reactions in cellular systems (He and Häder, 2002). The active oxygen species are highly deleterious for cell structures and functions (Hideg and Vass, 1996; He and Häder, 2002). In order to prevent the harmful effects caused by the stress, organism develops radical quenchers and antioxidants that provide protection by scavenging harmful radical or oxygen species (Mittler and Tel-Or, 1991; Middleton and Teramura, 1993). Detailed studies on the mechanisms of UV induced impairment of photosynthesis and antioxidant response have been predominantly conducted on crop plants (Borman, 1989; Jordan, 1996; Prasad et al., 2005), while comparative studies on these aspects in microorganisms particularly cyanobacteria are still scarce. Thus, in the present study an attempt has been made to compare the growth, photosynthetic pigments, photosynthetic activity, energy transfer efficiency within the antenna pigments of PS II, antioxidant enzymes and lipid peroxidation in three different cyanobacteria exposed to UV-B.

2. Materials and methods

2.1. Organisms, culture conditions and UV-B treatment

The cyanobacterial strains *Nostoc muscorum*, *Plectonema boryanum* and *Aphanothece* sp. were obtained on different solid agar media (1.5%) in Petri dishes (75 mm) from stock culture kept at the Ranjan Plant Physiology and Biochemistry Laboratory, University of Allahabad, India. *P. boryanum* and *Aphanothece* sp. were grown in nitrate containing BG-11 medium (Hugel et al., 1958), while *N. muscorum* was cultured in nitrogen free Chu-10 medium (Gerloff et al., 1950). The cultures were illuminated under fluorescent light of 72 µmol m⁻² s⁻¹ PAR at 26±2 °C with 14/10 h light and dark period. Exponentially grown cultures of required cell density (1.5 µg Chl a ml⁻¹) were placed in open Petri dishes occupying a depth of 2.5 mm and cells were exposed to UV-B radiation (0.4 W m⁻², simulating 15% ozone depletion at Varanasi, adjoining to Allahabad) for 15 and 60 min which correspond to 0.36 and 1.44 kJ m⁻², respectively. The source of UV-B radiation was single Philips UV-B (TL 40 W/12, The Netherlands) tube with its main output at 312 nm. During UV-B treatment, suspensions were given additional exposure of PAR (32 µmol m⁻² s⁻¹) and also gently agitated by magnetic stirrer to ensure uniform exposure. Radiation below 280 nm was eliminated by using the cellulose acetate filter and the radiation intensity was measured with a Power Meter (Spectra Physics, Model 407, A-2, USA).

2.2. Growth and photosynthetic pigments measurement

Growth experiments were conducted in liquid medium and the cultures were UV-B irradiated for 30, 60, 90 and 120 min which correspond to 0.72, 1.44, 2.16 and 2.88 kJ m⁻², respectively. Total protein content of UV-B irradiated and control cultures were estimated at regular intervals for 10 days according to the method of Lowry et al. (1951). Chlorophyll *a* and carotenoids were extracted in 80% acetone from treated and untreated cyanobacterial cells and the amount was estimated according to the method of Myers and Kritz (1955). Phycocyanin was extracted in 2.5 mM phosphate buffer (pH 7.0) after repeated freezing and thawing. The absorbance of transparent blue supernatant was recorded at 620 nm and phycocyanin content in each sample was calculated according to Blumwald and Tel-Or (1982).

2.3. Photosynthetic O₂ evolution and ¹⁴C- fixation

Oxygen yield and ¹⁴C-fixation were measured after 4th day of UV-B exposure (15 and 60 min). Photosynthetic oxygen evolution of UV-B treated and untreated cultures was determined by a Clark type O₂ electrode (Rank Brothers, U.K.) in a temperature controlled airtight reaction vessel illuminated with saturating light intensity (360 µmol m⁻² s⁻¹, PAR) at 28 °C and expressed as µmol O₂ evolved (mg Chl a⁻¹)⁻¹ h⁻¹. Photofixation of carbon was studied by the addition of 50 µl NaH¹⁴CO₃ (specific activity, 9.25 x 10⁴ Bq ml⁻¹) to the 5 ml of culture suspensions thereafter cultures were exposed to 360 µmol m⁻² s⁻¹ PAR at 28 °C for 5 min. The reaction was terminated by addition of 0.1 ml of 2 M HCl and the samples were flushed with air for 30 min to remove the dissolved ¹⁴CO₂. The radioactivity of ¹⁴C-incorporated in acid stable compounds was counted by LKB-1209, Rack Beta liquid scintillation counter. Rate of ¹⁴C-fxiation in sample is expressed as [S⁻¹ (µg Chl a⁻¹)⁻¹ h⁻¹].
2.4. Photosynthetic electron transport activities

Photosynthetic electron transport activities were measured in spheroplasts prepared by the method of Spiller (1980). Spheroplasts were suspended in a medium containing 0.5 M sucrose, 10 mM HEPES–NaOH buffer (pH 6.9), 5 mM K2HPO4, 10 mM MgCl2, 0.5% (W/V) BSA. Prior to assay of electron transport activity the spheroplasts were exposed to UV-B for 15 and 60 min in the presence of 32 µmol photon m⁻² s⁻¹ PAR at 28 °C. The electron transport activity of PS II and PS I in control and UV-B treated spheroplast suspensions was measured at 28 °C by providing a PAR of 360 µmol m⁻² s⁻¹ for 5 min. PS II activity was measured as O₂ evolution by using 1 mM p-BQ as an electron acceptor. PS II activity was also studied by recording the DCPIP photoreduction spectrophotometrically at 600 nm in the presence and absence of artificial electron donors diphenyl carbazide (DPC), manganese chloride (MnCl₂) and hydroxylamine (NH₂OH). PS I mediated electron transport rate was recorded by monitoring O₂ consumption in the reaction mixture containing 50 µM DCPIP, 1 mM ascorbate, 10 µM DCMU, 50 µM NaN₃ and 0.1 mM MV. In each assay spheroplasts equivalent to 3 µg Chl a ml⁻¹ were used.

2.5. Fluorescence spectra

Cells were exposed to UV-B for 15 and 60 min in the presence of 32 µmol photon m⁻² s⁻¹ PAR at room temperature. Immediately after treatments fluorescence spectra of UV-B treated and untreated cells were recorded after exciting at 514.5 nm by using Laser Spectrofluorometer (Spectra Physics, Model 2016, USA).

2.6. Assay of antioxidant enzymes

The activity of antioxidative enzymes was determined after 4th day of UV-B exposure (15 and 60 min). Catalase (EC 1.11.1.6) activity was determined by following O₂ release from dissociation of H₂O₂ for 1 min after the addition of 5 ml of 50 mM phosphate buffer (pH 7.0) containing 50 mM H₂O₂ to 1 ml of cell suspension in darkness (Egashira et al., 1989). Oxygen release due to enzymatic dissociation of H₂O₂ was measured by a Clark type O₂ electrode (Rank Brothers, UK) and the oxygen produced by enzymatic reaction was calculated after correction for autoproduction of oxygen from H₂O₂. One unit of catalase is the amount of enzyme producing 1 µmol O₂ min⁻¹ as described by Sgherri et al. (2001). To determine the activity of SOD (EC 1.15.1.1), test samples were harvested by centrifugation and homogenised at 4 °C in 100 mM EDTA phosphate buffer (pH 7.8). Supernatant obtained after centrifugation of the homogenate at 20,000 g for 30 min was used as a crude extract for SOD spectrophotometric assay of Giannopolitis and Ries (1977) using 3 ml of reaction mixture containing 1.3 mM riboflavin, 13 mM L-methionine, 0.05 M Na₂CO₃ (pH 10.2), 63 µM p-nitroblue tetrazolium chloride, and 0.1 ml of crude extract. Peroxidase (EC 1.11.1.7) activity in a reaction mixture (3 ml) containing 16 mM H₂O₂, 10 mM pyrogallol and crude extract (650 µg protein ml⁻¹) was determined spectrophotometrically according to the method of Gahagen et al. (1968) and the activity was measured as rise in optical density at 430 nm.

2.7. Measurement of malondialdehyde (MDA)

Thiobarbituric acid reactive substances production in test samples was measured after 4th day of UV-B treatment by the method of Heath and Packer (1968).

Fig. 1. Growth behaviour of Nostoc muscorum (a), Plectonema boryanum (b) and Aphanothece sp. (c) exposed to UV-B stress. Values are means ± SE (n=3).
2.8. Statistical analysis

Values presented in the text indicate mean values±S.E. of three replicates. The significance of differences between control and treated samples were analysed using the Student’s t-test at the level of significance of \( P<0.01 \) and \( P<0.05 \).

3. Results

3.1. Growth

The growth responses of three tested cyanobacteria *N. muscorum*, *P. boryanum* and *Aphanothece* sp. to UV-B was inhibitory and the effect varied with UV-B dose and cyanobacterial strains (Fig. 1a, b, c). *N. muscorum* did not show any growth for at least 4–6 days even after 30 min of UV-B exposure, and then growth started slowly and attained a value about 32% of control. Similar treatment in *Aphanothece* sp. exhibited comparable growth than that of control while the final growth yield in *P. boryanum* was 89% of control. Furthermore, the decreasing trend continued upon increasing exposure time as 120 min of UV-B exposure caused severe damage to cellular system (membrane integrity, light harvesting complex and activity of photosystems) in *N. muscorum* as there was only 16% final growth yield and in similar conditions there was considerable growth in *P. boryanum* (56%) and *Aphanothece* sp. (68%).

3.2. Photosynthetic pigments

Contents of chlorophyll *a* and phycocyanin in test cyanobacteria reduced considerably following UV-B exposure and the decrease was UV-B dose dependent (Fig. 2a, b, c). As a result of increased UV-B exposure time from 15 to 60 min, chlorophyll *a* and phycocyanin contents decreased in *N. muscorum* from 15 to 50% and 25 to 75%; in *P. boryanum* from 8 to 25% and 15 to 40%; in *Aphanothece* sp. from 5 to 15% and 10 to 12%, respectively. Compared to chlorophyll *a*, phycocyanin was severely affected as the ratio of phycocyanin to chlorophyll *a* declined with rising dose of UV-B (data not shown). Carotenoids showed varied response as UV-B exposure for 60 min caused a decrease of 35 and 19% in *N. muscorum* and *P. boryanum*, respectively while a similar treatment exhibited marginal rise (5%) in *Aphanothece* sp. (Fig. 2b).

3.3. Photosynthetic activity

Similar to growth and photosynthetic pigments, UV-B exposure for 15 min suppressed the whole cell oxygen yield by 14, 8 and 3% after 4th day of treatment and the yield further declined to 38, 24 and 9% in *N. muscorum*, *P. boryanum* and *Aphanothece* sp., respectively following 60 min of UV-B exposure (Table 1). Induced inhibition of PS II activity in spheroplasts exposed to UV-B varied significantly among three cyanobacterial strains as 60 min of UV-B exposure resulted in 50, 37 and 8% suppression of PS II activity in *N. muscorum*, *P. boryanum* and *Aphanothece* sp., respectively. In contrast to this, marginal decrease in PS I activity was observed following the similar dose of UV-B. In order to pinpoint the possible site of action of UV-B on PS II, the electron transport activity was estimated in the presence of artificial electron donors DPC, \( \text{NH}_2\text{OH} \) and \( \text{MnCl}_2 \) (Table 2). These electron donors restored the PS II activity partially, however, the degree of restoration

Fig. 2. Chlorophyll *a*, carotenoid and phycocyanin contents of *N. muscorum*, *P. boryanum* and *Aphanothece* sp. exposed to UV-B for 15 and 60 min. Chlorophyll *a* (*a*), carotenoids (*b*) and phycocyanin (*c*) in untreated controls were 1.07±0.02, 0.40±0.03 and 13.32±0.6 µg ml\(^{-1}\) in *N. muscorum*; 1.10±0.02, 0.33±0.02 and 7.37±0.04 µg ml\(^{-1}\) in *P. boryanum*; 1.12±0.02, 0.50±0.02 and 2.5±0.02 µg ml\(^{-1}\) in *Aphanothece* sp., respectively. Values are means±SE (n=3).
was different in cyanobacterial strains and also varied with UV-B exposure time. Compared to whole cell oxygen evolution, photofixation of CO₂ diminished more rapidly as there was 56, 32 and 15% reduction in carbon fixation after 60 min of UV-B exposure in *N. muscorum*, *P. boryanum* and *Aphanothece* sp., respectively (Table 1).

### 3.4. Fluorescence

The emission spectra of phycocyanin in tested cyanobacteria were depicted in Fig. 3a, b and c. The phycocyanin spectra of intact cells of these cyanobacteria exhibited an emission band approximately at 651 nm and the intensity of fluorescence emission declined with increasing UV-B exposure time in *N. muscorum* and *P. boryanum*. However, 15 min of UV-B exposure showed slight rise in emission peak in *Aphanothece* sp. Suppression in emission peak after 60 min of UV-B exposure was more pronounced in *N. muscorum* which was followed by *P. boryanum* and *Aphanothece* sp.

### 3.5. Lipid peroxidation

UV-B induced the formation of MDA indicating enhanced lipid peroxidation in cyanobacteria (Table 3). Content of MDA increased by 10, 7 and 3% with 15 min of UV-B exposure in *N. muscorum*, *P. boryanum* and *Aphanothece* sp., respectively. The content of MDA further increased with rising UV-B exposure time (60 min).

### 3.6. Antioxidant enzymes

The activity of antioxidant enzymes in cyanobacteria such as SOD, CAT and POD was enhanced at 15 and 60 min of UV-B exposure (Table 3). The activity of SOD, CAT and POD increased by 13, 8 and 7% in *N. muscorum*, 18, 12 and 9% in *P. boryanum* and 19, 13 and 11% in *Aphanothece* sp., respectively.

### Table 1

| Experimental organisms | UV-B exposure (min) | Phosynthetic O₂ yield [μmol O₂ (mg Chl a⁻¹ h⁻¹)] | ¹⁴C-fixation [µg Chl a⁻¹ h⁻¹] | Electron transport rate [µmol O₂ (mg Chl a⁻¹ h⁻¹)] |
|------------------------|---------------------|---------------------------------|------------------|-------------------------------|
| *N. muscorum*          | 0                   | 207±6.0                         | 1190±12          | 257±2                         | 490±3 |
|                        | 15                  | 178±4.2 (14)                    | 963±7 (19)       | 193±2 (25)                    | 475±2 (3) |
|                        | 60                  | 128±4.0 (38)                    | 523±7 (56)       | 128±1 (50)                    | 470±2 (4) |
| *P. boryanum*          | 0                   | 258±4.4                         | 1350±13          | 290±2                         | 620±4 |
|                        | 15                  | 237±4.0 (8)*                    | 1146±10 (15)     | 255±7 (12)                    | 614±2 (1)* |
|                        | 60                  | 196±3.6 (24)                    | 918±7 (32)       | 184±2 (37)                    | 608±2 (2)* |
| *Aphanothece* sp.      | 0                   | 221±4.0                         | 1316±12          | 270±3                         | 580±3 |
|                        | 15                  | 214±2.0 (3)*                    | 1223±8 (7)       | 256±2 (5)                     | 574±3 (1)* |
|                        | 60                  | 201±2.2 (9)                     | 1118±8 (15)      | 248±2 (8)                     | 568±3 (2)* |

Means±SE. Values in parenthesis are [%] decrease. All the treatments are significantly different (*P*<0.01, *P*<0.05) from control (Student’s *t-*test). ns = not significant.

### Table 2

Effect of various exogenous electron donors on PS II activity (H₂O→DCPIP) in UV-B treated spheroplasts of cyanobacteria.

| Experimental organisms | UV-B exposure (min) | Without donors | With donors | DPC | NH₃OH | MnCl₂ |
|------------------------|---------------------|----------------|-------------|-----|-------|-------|
| *N. muscorum*          | 0                   | 65±0.7         | 95±1.5      | 79±1.2 | 68±1.0 |
|                        | 15                  | 49±0.5 (25)    | 81±0.7 (15) | 66±0.6 (16) | 54±0.5 (21) |
|                        | 60                  | 34±0.4 (48)    | 56±0.5 (41) | 46±0.4 (42) | 39±0.3 (43) |
| *P. boryanum*          | 0                   | 80±1.0         | 103±1.5     | 92±1.2 | 87±1.0 |
|                        | 15                  | 70±0.8 (13)    | 101±1.0 (2)* | 88±0.9 (4)* | 82±0.8 (6) |
|                        | 60                  | 51±0.5 (36)    | 88±0.8 (15) | 75±0.7 (18) | 69±0.6 (21) |
| *Aphanothece* sp.      | 0                   | 75±0.7         | 90±1.3      | 85±1.2 | 80±1.0 |
|                        | 15                  | 70±0.7 (7)     | 89±0.9 (1)* | 83±0.8 (2)* | 78±0.7* (3) |
|                        | 60                  | 66±0.6 (12)    | 86±0.8 (4)* | 81±0.8 (5)* | 74±0.7 (8) |

Means±SE. Values in parenthesis are [%] decrease. All the treatments are significantly different (*P*<0.01, *P*<0.05) from control (Student’s *t-*test). ns = not significant.
N. muscorum, P. boryanum, and Aphanothece sp. The growth responses of these cyanobacteria to UV-B stress showed considerable differences probably due to different degree of damage caused by UV-B directly or indirectly on DNA, proteins, and photosynthetic apparatus (Melis et al., 1992; Friso et al., 1994; Callaghan et al., 2004). The occurrence of some UV protecting pigments and UV sunscreen role of mycosporine (MAA) like compounds have been demonstrated in several cyanobacterial strains (Castenholz, 1997; Adhikary and Sahu, 1998; Sinha and Häder, 2008) and varied levels of these compounds in the cells may be one of the reasons for differential sensitivity of cyanobacteria. Besides this, the differential responses of these cyanobacteria to UV-B could also be explained on the basis of morphological features such as sheath, antioxidants and DNA repairing system (He and Häder, 2002; Singh et al., 2008; Sinha and Häder, 2008).

4. Discussion

The present study deals with UV-B induced changes in growth, pigments, and photosynthetic electron transport activities, photofixation of $^{14}$CO$_2$ and energy transfer efficiency in phycobilisomes, antioxidant enzymes and lipid peroxidation of three morphologically different cyanobacteria N. muscorum, P. boryanum and Aphanothece sp. The growth responses of these cyanobacteria to UV-B stress showed considerable differences probably due to different degree of damage caused by UV-B directly or indirectly on DNA, proteins, and photosynthetic apparatus (Melis et al., 1992; Friso et al., 1994; Callaghan et al., 2004). The occurrence of some UV protecting pigments and UV sunscreen role of mycosporine (MAA) like compounds have been demonstrated in several cyanobacterial strains (Castenholz, 1997; Adhikary and Sahu, 1998; Sinha and Häder, 2008) and varied levels of these compounds in the cells may be one of the reasons for differential sensitivity of cyanobacteria. Besides this, the differential responses of these cyanobacteria to UV-B could also be explained on the basis of morphological features such as sheath, antioxidants and DNA repairing system (He and Häder, 2002; Singh et al., 2008; Sinha and Häder, 2008).

UV-B induced damaging response on photosynthetic pigments in these cyanobacteria varied significantly (Fig. 2a, b, c). Similar UV-B induced differential effect on photosynthetic pigments was also noticed in brown and blue green strains of Nostoc spongiforme where blue green strain showed more strong effect (Tyagi et al., 1992). It was suggested that the damaging effect on photosynthetic pigments was due to the bleaching caused by UV-B radiation or through active oxygen mediated peroxidation (Nultsch and Agel, 1986). The increase in carotenoid contents in response to UV-B in Aphanothece sp. may be attributed to their protective role in scavenger of singlet oxygen (Middleton and Teramura, 1993; Vincent and Quesada, 1994). Singlet oxygen is generally produced during normal photosynthesis and its formation may further increase under stress including UV-B radiation (Xue et al., 2005). Among three photosynthetic pigments the severe effect on phycocyanin in these cyanobacteria was in coherence with earlier findings (Döhler et al., 1986; Tyagi et al., 1992). The heavy loss of phycocyanin might have occurred due to direct interaction of UV-B with phycocyanin, localized on the outer surface of thylakoid membrane. Phycocyanin being proteaceous in nature may directly absorb radiation in UV-B region (280 nm and above) and get damaged. Furthermore, AOS induced protein peroxidation may also cause damaging effect on phycocyanin.

The reduction in whole cell photosynthetic oxygen yield of these cyanobacteria could be linked to direct interaction of UV-B with components of photosynthetic electron transport chain mainly PS II (Table 1) and energy transfer efficiency of phycocyanin (Fig. 3a, b, c) and an indirect effect on CO$_2$ fixation ability (Table 1). PS II associated with oxygen evolving complex has been characterized as the most labile component of photosynthetic electron transport chain against stress (Renger et al., 1989; Prasad and Zeeshan, 2004; Prasad et al., 2005). The partial restoration in PS II activity by artificial electron donors suggested that the inhibition of PS II activity occurred as a result of interruption of electron flow at water oxidation site in P. boryanum after 15 min of UV-B exposure, and in Aphanothece sp. after 60 min of UV-B exposure (Table 2). Compared to this less restoration in PS II activity after 15 min of UV-B exposure in
**Table 3**

Effect of UV-B on antioxidant enzymes and lipid peroxidation in cyanobacteria.

| Organisms  | UV-B exposure (min) | Enzyme activity | Lipid peroxidation |
|------------|---------------------|-----------------|-------------------|
|            |                     | Superoxide dismutase [unit (mg protein)] | Catalase [unit (mg protein)] | Peroxidase [change in OD430 (mg protein)⁻¹ min⁻¹] | MDA content nmol (mg protein)⁻¹ |
|            |                     | [¹] | [¹] | [¹] | [¹] |
| *N. muscorum* | 0                  | 8±0.2 | 4.8±0.04 | 3.0±0.01 | 1.90±0.05 |
|            | 15                 | 9±0.2 (13)* | 5.2±0.04 (8) | 3.2±0.02 (7) | 2.10±0.05 (10)* |
|            | 60                 | 10±0.3 (25) | 5.5±0.05 (15) | 3.3±0.02 (10) | 2.32±0.06 (22) |
| *P. boryanum* | 0                  | 14±0.3 | 8.5±0.05 | 9.0±0.03 | 1.60±0.02 |
|            | 15                 | 17±0.4 (18) | 9.5±0.06 (12) | 9.8±0.04 (9) | 1.71±0.03 (7)* |
|            | 60                 | 19±0.5 (34) | 10.5±0.07 (24) | 10.5±0.05 (17) | 1.84±0.04 (15) |
| *Aphanothece* sp. | 0                  | 17±0.4 | 9.7±0.05 | 13.0±0.04 | 1.40±0.02 |
|            | 15                 | 21±0.5 (24) | 11.6±0.06 (20) | 15.0±0.06 (15) | 1.45±0.02 (3)* |
|            | 60                 | 26±0.5 (53) | 13.4±0.08 (38) | 17.0±0.07 (31) | 1.52±0.03 (9)* |

Means±SE. Values in parenthesis are [%] increase. All the treatments are significantly different (*P<0.01, *P<0.05) from control (Student’s t-test). ns = not significant.

*N. muscorum* and 60 min in *P. boryanum* pointed out that the extent of inhibition had extended to PS II reaction center. UV-B induced suppression in PS II activity might have occurred due to the damage caused on the oxygen evolving complex and also on D1 and D2 polypeptides of PS II reaction center. It has been reported that the quantum yield for D1 degradation is highest in D1 and D2 polypeptides of PS II reaction center. It has been explained on the basis of reduced supply of ATP and NADPH due to inhibitory action of UV-B on photosynthetic electron transport reactions (Table 1) by arresting the electron flow (Prasad et al., 2005).

Furthermore, UV-B induced arrest in electron flow (PS II) probably enhanced the generation of peroxide which could not be detoxified by even enhanced activity of POD and CAT thus, excessive accumulation of hydrogen peroxide may lead to the increased rate of lipid peroxidation causing severe damage to cellular membranes (Table 3). Tyagi et al. (1992) have also demonstrated heavy reduction in ¹⁴C-fixation in two forms of *N. spongiaforme* after 60 min of UV-B exposure. The greater sensitivity of ¹⁴C-fixation could also be correlated with direct effect of UV-B on Rubisco subunits as suggested in dinoflagellate *Proorocentrum micans* (Lesser et al., 1994). Severe loss of Rubisco subunits in marine macroalgae following UV-B exposure has also been demonstrated and Rubisco enzyme was shown to be the primary site of UV-B radiation rather than photosynthetic electron transport chain (Bischof et al., 2000).

The exposure of UV-B arrested the electron flow at the oxidation site of PS II (Tables 1 and 2) that might have resulted into the formation of active oxygen species which in turn poses an intrinsic threat to cellular organization because many components within the cells are susceptible to oxidative damage (Dai et al., 1997; He and Häder, 2002). In the present study UV-B treatments has led to a significant increase of the MDA content in *N. muscorum*, *P. boryanum* and *Aphanothece* sp. (Table 3). The results indicate high degree of lipid peroxidation in *N. muscorum* and low degree in *Aphanothece* sp. Increased MDA content in cyanobacteria was probably because of oxidative degradation of polyunsaturated fatty acids in membranes (Girotti, 1990). Chris et al. (2006) observed an increase in MDA content in *Cylindrospermum* sp. following UV-B exposure. Increased content of MDA following lipid peroxidation indicates oxidative stress in cyanobacteria. During oxidative stress active oxygen species (AOS) particularly H₂O₂ and O₂ triggered the activity of several antioxidative enzymes such as SOD, CAT and POD. High activity of these antioxidative enzymes in cyanobacteria could be linked with stress tolerance efficiency. The greater increase in the activity of SOD, CAT and POD in *Aphanothece* sp. followed by *P. boryanum* and *N. muscorum* to UV-B stress indicates the high efficiency of stress tolerance in *Aphanothece* sp. (Table 3). Earlier studies have also demonstrated the increased activity of these enzymes to UV-B exposure in plants (Kondo and Kawashima, 2000).
The present study concludes that the three cyanobacteria exhibited different degrees of sensitivity to UV-B showing maximum effect on *N. muscorum* followed by *P. boryanum* and *Aphanothece* sp. The greater tolerance in *Aphanothece* sp. to UV-B could be explained on the basis of thick sheath. Since sheath is supposed to be site of production of UV-B protectant compounds and thus, sheath probably reduces UV-B penetration inside the cells, therefore, sheathed cyanobacteria might be tolerant in comparison to one that has less prominent sheath (Singh et al., 2008; Sinha and Häder, 2008). In another finding the measurements of UV-B penetration in higher plant leaves through epidermal layer into the mesophyll using a fiber-optic microprobe have confirmed the importance of absorbing material in their epidermal layer (Bormann and Vogelmann, 1991). Another reason for greater tolerance of *Aphanothece* sp. was probably due to the efficient antioxidant system and thereby, exhibiting less inhibitory effect on photosynthetic pigments, oxygen yield, PS II activity, energy transfer efficiency in the phycobilisomes and 14C-fixation ability. A lower lipid peroxidation with high capacity of scavenging systems could probably explain the ability of *Aphanothece* sp. to grow in paddy fields at enhanced UV-B exposure than *P. boryanum* and *N. muscorum*, which appear to be sensitive.

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