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Research article

Differential effect of UV-B radiation on growth, oxidative stress and ascorbate–glutathione cycle in two cyanobacteria under copper toxicity

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ABSTRACT

Effects of low (UV-B_L; 0.1 μmol m^{-2} s^{-1}) and high (UV-B_H; 1.0 μmol m^{-2} s^{-1}) fluence rates of UV-B radiation on growth, oxidative stress and ascorbate–glutathione cycle (AsA–GSH cycle) were investigated in two cyanobacteria viz. *Phormidium foveolarum* and *Nostoc muscorum* under copper (2 and 5 μM) toxicity after 24 and 72 h of experiments. Cu at 2 and 5 μM and UV-B_H irradiation decreased growth in both the organisms and the effect was more pronounced in *N. muscorum*. Superoxide radical (SOR) and hydrogen peroxide (H_2O_2) productions were significantly enhanced by Cu and UV-B_H which was accompanied by accelerated lipid peroxidation (malondialdehyde; MDA) and protein oxidation (reactive carbonyl groups; RCG). The components of AsA–GSH cycle, i.e. ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascobate reductase (MDHAR) and dehydroascorbate reductase (DHAR) activities as well as total ascorbate and glutathione contents and their reduced/oxidized ratios were decreased considerably by Cu and UV-B_H. Further, combined treatments of Cu and UV-B_H exacerbated damaging effects in both the cyanobacteria. Unlike UV-B_H, UV-B_L irradiation rather than damaging cyanobacteria caused alleviation in Cu-induced toxicity by down-regulating the levels of SOR, H_2O_2, MDA and RCG due to enhanced activity of APX, GR, MDHAR and DHAR, and contents of ascorbate and glutathione. Results revealed that UV-B radiation at low fluence rate (UV-B_L) stimulated protective responses in both the organisms under Cu toxicity while UV-B_H irradiation caused damage alone as well as together with Cu, and the components of AsA–GSH cycle play significant role in these responses.

1. Introduction

Copper (Cu) is an essential micronutrient with a requirement among the lowest of all elements [1]. However, at higher concentration it causes phytotoxic effects [2]. Cu concentration in agricultural soils and water bodies may reach appreciably high as a consequence of excess application of organic fertilizers and Cu-containing fungicides [1,2]. Cu being a transitional element at higher concentration causes oxidative stress in cells by generating reactive oxygen species (ROS) [3]. Besides this, Cu can interrupt the electron flow during photosynthetic and respiratory processes and thus induces the generation of ROS. The excess ROS react with biomolecules such as lipids, nucleic acids and proteins hence leads to altered fluidity of membrane, loss of enzyme function and genomic damage [2,3,4]. Cu can also induce formation of highly reactive hydroxyl radical (•OH) from superoxide radical ($O_2^{\bullet -}$) or hydrogen peroxide (H_2O_2) via Haber–Weiss reaction [3]. The ascorbate–glutathione cycle (AsA–GSH cycle) is one of the central and efficient antioxidant systems for removal of ROS and the maintenance of cellular redox balance [1,5,6]. AsA–GSH cycle comprises of various enzymes such as ascorbate peroxidase (APX; EC 1.11.1.11), monodehydroascorbate reductase (MDHAR; EC 1.6.5.4), dehydroascorbate reductase (DHAR; EC 1.8.5.1) and glutathione reductase (GR; EC 1.6.4.2), and metabolites, viz. reduced ascorbate (AsA), monodehydroascorbate (MDHA), dehydroascorbate (DHA), reduced (GSH) and oxidized (GSSG) glutathione [5,7]. The AsA–GSH cycle is the recycling pathway of ascorbate and glutathione and plays important role in maintaining the reduced forms of ascorbate and glutathione in cell thus protects plants against oxidative stress [1,5].

Depletion of the stratospheric ozone layer due to the anthropogenic activities is resulted into an increased UV-B radiation (280–320 nm) on the Earth's surface and causing damage to the biological system. In recent years, studies have demonstrated that low UV-B fluence rate (<1.0 μmol m^{-2} s^{-1}) is capable of promoting metabolic and developmental changes such as biosynthesis of phenolics and photomorphogenesis in higher

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plants [8–10]. It has also been shown that low UV-B fluence rate responses involve specific receptors and appear to be photoregulatory. Hence, low UV-B may function as informational signal that exerts protective effects. On other hand, damaging effects of enhanced UV-B radiation on plants including cyanobacteria are well established [8,11,12]. High UV-B fluence rate ($>1.0\ \mu mol\ m^{-2}\ s^{-1}$) responses have been shown to be mediated by DNA damage signaling by producing excess ROS, which cause damage to DNA, proteins, membranes and lipids, and do not involve specific receptors [8,10].

Cyanobacteria are major biomass producers in aquatic ecosystems and represent more than 50% of the biomass in several ecosystems. Further, their inherent capacity to fix molecular nitrogen into ammonia has made them ecologically important for rice-growing countries in recent years [12]. Cyanobacteria may have the ability to grow even up to 7 m to the depth of water where the intensity of light (photosynthetically active radiation; PAR) is quite low (8–10 μmol photons $m^{-2}\ s^{-1}$) [13]. These organisms can occupy different habitats as well as variable depths of water including paddy field hence they may receive varied levels of UV-B radiation and respond accordingly. It has been reported that UV-B can penetrate up to several centimeters to >10 m in clear water [14,15] while Hurtubise and Havel [16] noticed that in eutrophic pond UV-B can have only 10% of irradiance up to 30 cm depth and further they have recorded variable energy levels (0.042–0.84 $W\ m^{-2}$) of UV-B radiation. Recently, it has been demonstrated that UV-B-induced responses in plants may depend on UV-B fluence rates [8,10]. Thus, this study was undertaken to investigate whether low (0.1 $\mu mol\ m^{-2}\ s^{-1}$ for 4 h; 0.038 $W\ m^{-2}$; simulating UV-B radiation at 30 cm depth of eutrophic pond) and high (1.0 $\mu mol\ m^{-2}\ s^{-1}$ for 4 h; 0.38 $W\ m^{-2}$; simulating ambient UV-B radiation at study place) fluence rates of UV-B affect the growth, and AsA–GSH cycle performance differently in two cyanobacteria viz. *Phormidium foveolarum* and *Nostoc muscorum* exposed to Cu toxicity (2 and 5 μM). Besides the present environmental problem, this study also focuses on the survival strategy of cyanobacteria, one of the pioneering photoautotrophs to withstand against dual stress, i.e. Cu and UV-B during the early period of evolution. It has been proposed that following the onset of oxygenic photosynthesis during early period of evolution, the reducing environment changed into oxidized form; hence, iron found in ionic form in water bodies was replaced by copper to continue biochemical processes and the level of copper increased considerably in due course of time [17].

2. Results

2.1. Growth

Cu (2 and 5 μM) treatment caused deleterious effect on *N. muscorum* and *P. foveolarum*. Cu at 2 and 5 μM significantly ($P < 0.05$) decreased dry mass in *P. foveolarum* by 19 and 42% and in *N. muscorum* by 32 and 51%, respectively, compared to the control samples after 24 h of experiment (Fig. 1A and C). Under similar condition, dry mass declined by 18 and 33% in *P. foveolarum* and 21 and 43% in *N. muscorum*, respectively, after 72 h of experiment (Fig. 1B and D). The high fluence rate (UV-B_H) of UV-B suppressed ($P < 0.05$) the growth by 18% in *P. foveolarum* and by 26% in *N. muscorum* after 24 h of experiment (Fig. 1A and C). Cu and UV-B_H together further exacerbated ($P < 0.05$) the damaging effect (Fig. 1A–D). After 24 h, maximum reduction in growth was observed in *N. muscorum* under 5 μM Cu and UV-B_H combination, and after 72 h of experiment the decline in growth continued. However, it was appreciably less than those recorded after 24 h (Fig. 1C and D). The low fluence rate of UV-B (UV-B_L) did not inhibit ($P < 0.05$) the growth in both the cyanobacteria, and in combination (Cu + UV-B_L) the toxic effect induced by Cu on growth was alleviated significantly in comparison to Cu alone treated cells (Fig. 1A–D).

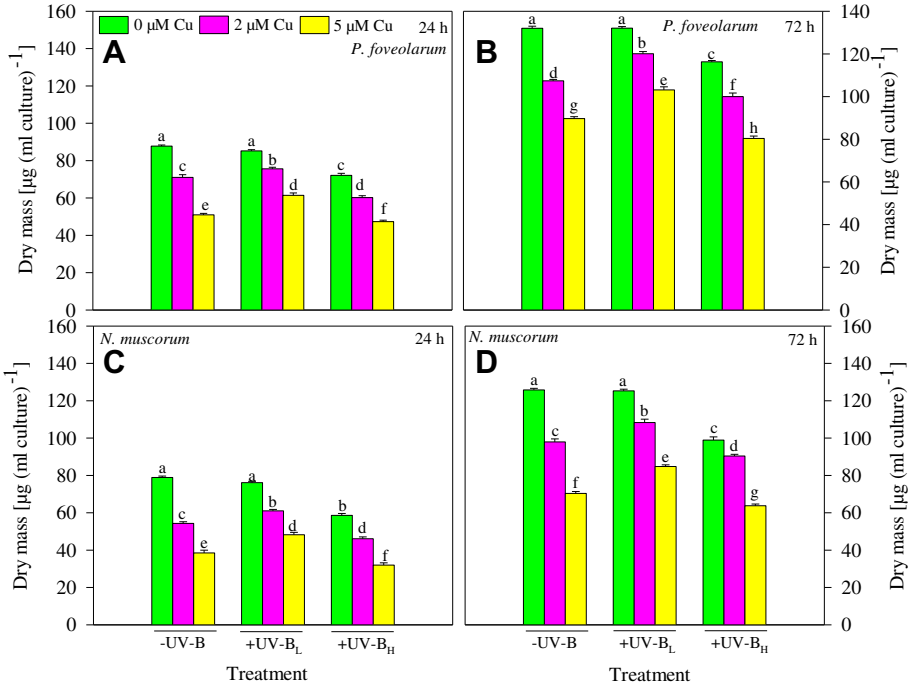

Fig. 1. Effect of low (UV-B_L) and high (UV-B_H) fluence rates of UV-B radiation on dry mass accumulation in *P. foveolarum* (A and B) and *N. muscorum* (C and D) exposed to Cu for 24 and 72 h. Data are means ± standard error of six replicates. Bars followed by different letter show significant difference at $P < 0.05$ significance level according to the Duncan's multiple range test.