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Kinetin alleviates chromium toxicity on growth and PS II photochemistry in *Nostoc muscorum* by regulating antioxidant system

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ABSTRACT

The present study was undertaken to evaluate the metal toxicity alleviating effects of kinetin (KN, 10 nM) on growth, photosynthetic pigments and photochemistry of PS II in the cyanobacterium *Nostoc muscorum* exposed to chromium (Cr^{VI}) stress (100 and 150 μM). Chromium declined growth, photosynthetic pigments (chlorophyll *a*, phycocyanin and carotenoids), photosynthetic oxygen evolution rate and parameters of fluorescence kinetics (ϕP_0, F_V/F_0, ϕE_0, Ψ_0 and PI_{ABS} except F_0/F_V) in concentration dependent manner, while stimulating effects on respiration, energy flux parameters (ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC), oxidative stress biomarkers i.e., superoxide radical (SOR), hydrogen peroxide (H_2O_2) and lipid peroxidation (TBARS contents) and antioxidative enzymes: superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione-*S*-transferase (GST), were observed. However, upon addition of KN in the growth medium an alleviating effect against chromium induced toxicity on growth, photosynthetic pigments and photochemistry of PS II was recorded. This had occurred due to substantial reduction in levels of oxidative stress biomarkers: SOR, H_2O_2 and TBARS contents with concomitant rise in activity of antioxidative enzymes: SOD, POD, CAT and GST and appreciable lowering in the cellular accumulation of chromium. The overall results demonstrate that KN application significantly alleviated chromium induced toxicity on growth performance of the cyanobacterium *N. muscorum* due to significant improvement in photosynthetic pigments and photochemistry of PS II by up-regulating the activity of antioxidative enzymes, and declining cellular accumulation of chromium. Furthermore, Cr induced toxicity at lower dose (100 μM) was found to be ameliorated more efficiently in *N. muscorum* following supplementation of KN.

1. Introduction

Cyanobacteria, gram negative oxygenic photosynthetic prokaryotes are considered as major components of microbial population, dominate in wetland soils especially in paddy fields (Zehr, 2011). Among the microbial population cyanobacteria are major contributors of global biomass as they are capable of fixing atmospheric nitrogen into ammonia and simultaneously perform photofixation of carbon dioxide (Salma et al., 2014). Due to this unique feature, cyanobacteria since time immemorial are being used as a bio-fertilizer in the paddy fields to enhance the productivity. In recent years, in rice growing countries such as India, Srilanka, China etc. cyanobacteria are considered as a cost effective bio-fertilizer which may improve the physio-chemical properties of the soil hence substantially increase its water-holding capacity and mineral nutrient status (Singh et al., 2016). Other than this, in recent years efforts are being made to exploit them as potential source of food and several valuable medicines by pharmaceutical industries (Pangestuti and Kim, 2011).

Industrialization and agricultural activities have resulted in hasty discharge of toxic metals such as chromium (Cr), lead (Pb), cadmium (Cd), nickel (Ni) and arsenic (As) etc. in the environment hence posing countless threats to natural habitats of cyanobacteria (Maksymiec, 2007). Chromium (Cr), the seventh most abundant metal in the earth's crust, is a toxic as well as carcinogenic element and exists in two redox forms i.e. hexavalent chromate (Cr^{VI}) and trivalent chromite (Cr^{III}) (Ashraf et al., 2017). In two forms, Cr^{VI} is predominant in the environment due to its high solubility, mobility and rapid permeation across the biological membranes and thus causing strong damaging effects on biological systems (Viti et al., 2014). The safe limit of Cr^{VI} in the aquatic ecosystem has been reported to be 1 μg L^{-1} (Zayed and Terry, 2003). However, in the effluents released from paints, metal finishes, steel manufacturing, tanning process in leather industries, alloy cast iron, and chrome plating industries (Ashraf et al., 2017) its concentration may even greater than 5 g L^{-1}. According to Dutta et al. (2017), 32.04 ± 1.60 mg/kg soil Cr^{VI} reported in the soil of Sunderbans (India) where rice is grown as a staple food crop. In India, rice

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fields are often irrigated with Cr contaminated water that negatively affects the growth by adversely impacting pigment bio-synthesis, several sites of photosynthetic electron transport system including efficiency of photosystem II (PS II) (Ali et al., 2006; Gupta et al., 2014) and inactivating the nitrogenase enzyme (Kiran et al., 2016) and thus causing nitrogen imbalance in the paddy fields. Furthermore, study also demonstrated that Cr^{VI} can induce oxidative stress by enhancing the production of reactive oxygen species (ROS) (Gupta and Ballal, 2015) which mediate the oxidative deterioration of lipids, proteins and DNA (Singh et al., 2016). Hence, chromium contamination in the aquatic ecosystem is a serious environmental concern and imposes toxicity to primary producers. In response to oxidative stress every organism possesses an array of defense mechanism that involves enzymatic and non-enzymatic antioxidants (Gupta and Ballal, 2015; da Costa et al., 2016), for maintaining the level of ROS in the cell.

Plants including microorganisms protect themselves from heavy metal toxicity by producing phytohormones that act as signalling molecules and play a prominent role in growth and development (Hunt et al., 2011). Besides this, management of the aquatic vegetation by using natural as well as synthetic plant hormones may be a key strategy in maintaining the micro-flora of aquatic ecosystem. Among various plant growth regulators; cytokinins are of great importance that enhance the cell division, chlorophyll biosynthesis, regeneration of proliferating shoots in plants, etc. (Hwang et al., 2012). Kinetin (KN) is one of the artificial cytokinins that has been reported to improve the growth of crop plants under adverse environmental conditions such as salinity (Wu et al., 2012) and metal stress (Singh and Prasad, 2014). Regarding the role of exogenous application of phytohormones in managing abiotic stress, several studies have been carried out in plants and seaweeds, but there is a need to perform these investigations on cyanobacteria that have high potential of serving sustainable economy in near future. The objective of the present study was to understand the effect of exogenously supplemented KN on physiological and biochemical constituents of *Nostoc muscorum* exposed to Cr^{VI} and to our knowledge this is the first study where *Nostoc muscorum* was used as a model organism to analyse the role of exogenous application of KN under Cr toxicity.

2. Materials and methods

2.1. Growth conditions and treatments

The homogenous and axenic cultures of *Nostoc muscorum* were grown in BG-11 medium at 25 ± 2 °C under 75 μmol photons m^{-2} s^{-1} provided by white fluorescent tubes (Osram L 40 W/25–1) with a 14:10 h of light:dark regimes in a temperature-controlled culture room. All the experiments were performed with cultures growing in early exponential phase. Hexavalent chromium (Cr^{VI}) as a potassium dichromate ($K_2Cr_2O_7$) was used at 10–300 μM concentrations for screening experiments, and on the basis of this the two doses i.e., 100 and 150 μM of Cr^{VI} were selected which were found to inhibit the growth of *N. muscorum* by 10% (LC_{10}) and 30% (LC_{30}), respectively. Further, screening experiments for KN were also performed at 1, 3, 5, 10 and 20 nM and stimulatory concentration of KN i.e. 10 nm enhanced the growth by 15% was selected for detailed study. For preparation of stock solution, the required quantity of KN was dissolved in 1 ml of acetone and the final volume was maintained up to 25 ml by the addition of BG-11 medium. The experimental setup consisted of six combinations i.e. control (without Cr^{VI} and KN), 100 μM Cr^{VI}, 150 μM Cr^{VI}, control + KN, 100 μM Cr^{VI} + KN and 150 μM Cr^{VI} + KN. There were three replicates (n = 3) for each treatment and all the parameters were analysed after 96 h of experiment.

2.2. Measurement of growth

For estimation of growth, culture absorbance of *N. muscorum* treated with KN alone and in combination with Cr^{VI} was recorded at 750 nm by using double beam UV–visible spectrophotometer (Shimadzu, Japan). For dry weight measurement cultures were centrifuged and pellets were oven dried at 60–70 °C for 3 days.

2.3. Estimation of chromium accumulation

For the estimation of intracellular accumulation of Cr^{VI}, treated cyanobacterial cells (80 ml) were harvested, centrifuged and washed several times with 1 mM EDTA to remove surface bound metal. Further, cells were re-suspended in chilled phosphate buffer for 10 min to remove the apo-plastic Cr^{VI}, centrifuged and pellets were oven dried at 60–70 °C for 3 days. Dried cyanobacterial cells were then digested after adding 5 ml of tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in ratio of 5:1:1) at 80 °C until a transparent solution obtained. After cooling, the digested sample was finally maintained up to 5 ml with double distilled water. Concentrations of total Cr^{VI} in digested samples were estimated by using atomic absorption spectrometer (iCE 3000 series, Model 3500 AAS, Thermo scientific, UK). The instrument was calibrated by using standard solutions of Cr^{VI}.

2.4. Measurement of photosynthetic pigments

The amounts of chlorophyll *a* (Chl *a*) and carotenoids (Car) from treated and untreated samples were quantified by following the method of Porra et al. (1989) and Goodwin (1954) respectively. The pigments were extracted in methanol (100%) and the absorbance of the extract for Chl *a* and Car was recorded spectrophotometrically at 665 nm and 450 nm, respectively. The phycocyanin (Phy) was extracted in potassium phosphate buffer (pH 7.0) prior treated with toluene and the absorbance of the extract was recorded at 620 nm by following the method of Blumwald and Tel-Or (1982).

2.5. Measurement of whole cell O_2 evolution and respiration

Treated and untreated cells were used for measurement of photosynthetic oxygen evolution and respiration by using Clark type oxygen electrode (Digital Oxygen System, Model-10, Rank Brothers, UK). The cells were transferred into the temperature controlled air tight reaction vessels of O_2 electrode in the presence of light provided by projector lamp (360 μmol photon m^{-2} s^{-1}; PAR) for 3 min and the rate of oxygen evolution (photosynthesis) was measured. For the measurement of respiratory activity oxygen consumption was recorded in the darkness. Photosynthetic and respiratory rates were expressed as μmol O_2 evolved (mg^{-1} Chl *a*) h^{-1} and μmol O_2 consumed (mg^{-1} Chl *a*) h^{-1}, respectively.

2.6. Measurement of chlorophyll a fluorescence kinetics (JIP-test)

For the assessment of efficiency of PS II: chlorophyll *a* fluorescence was determined in 30 min dark-adapted treated and untreated cultures by using hand held fluorometer (AquaPen AP 100, Photon System Instruments, Czech Republic). The fluorescence parameters: quantum yield of primary photochemistry (F_v/F_m or ϕP_0), F_v/F_0, F_0/F_v, maximum quantum efficiency of PS Π photochemistry (Ψ_0), yield of electron transport per trapped exciton (ϕE_0), performance index (PI_{ABS}) of PS II and the energy fluxes per reaction centre (ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC) were analysed according to Strasser et al. (2000). Blue excitation light at 455 nm is intended for chlorophyll excitation, i.e., for measuring chlorophyll *a* fluorescence in algal cultures.

2.7. Estimation of oxidative biomarkers and indices of damage

Superoxide radicals (SOR; $O_2^{\bullet-}$) content in treated and untreated samples was determined following the method of Elstner and Heupel (1976) based upon formation of NO_2^- from hydroxylamine in the