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Amelioration of arsenic toxicity in rice: Comparative effect of inoculation of *Chlorella vulgaris* and *Nannochloropsis* sp. on growth, biochemical changes and arsenic uptake

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ABSTRACT

The present study was conducted to assess the responses of rice (*Oryza sativa* L. var. Triguna) by inoculating alga; *Chlorella vulgaris* and *Nannochlropsis* sp. supplemented with As(III) (50 μM) under hydroponics condition. Results showed that reduced growth variables and protein content in rice plant caused by As toxicity were restored in the algae inoculated plants after 7 d of treatment. The rice plant inoculated with *Nannochloropsis* sp. exhibited a better response in terms of increased root, shoot length and biomass than *C. vulgaris* under As(III) treatment. A significant reduction in cellular toxicity (thiobarbituric acid reactive substances) and antioxidant enzyme (SOD, APX and GR) activities were observed in algae inoculated rice plant under As(III) treatment in comparison to uninoculated rice. In addition, rice treated with As(III), accumulated 35.05 mg kg^{-1} dw arsenic in the root and 29.96 mg kg^{-1} dw in the shoot. However, lower accumulation was observed in As(III) treated rice inoculated with *C. vulgaris* (24.09 mg kg^{-1} dw) and *Nannochloropsis* sp. (20.66 mg kg^{-1} dw) in the roots, while in shoot, it was 20.10 mg kg^{-1} dw and 11.67 mg kg^{-1} dw, respectively. Results demonstrated that application of these algal inoculum ameliorates toxicity and improved tolerance in rice through reduced As uptake and modulating antioxidant enzymes. Thus, application of algae could provide a low-cost and eco-friendly mitigation approach to reduce accumulation of arsenic in edible part of rice as well as higher yield in the As contaminated agricultural field.

1. Introduction

Inorganic arsenic (iAs) is a toxic groundwater contaminant of geogenic origin, particularly in large deltas and along major rivers in the deprived regions of South and East-Asia (Sharma et al., 2014). Arsenic is present predominantly as arsenate (As(V)) in the aerobic environment and as arsenite (As(III)) under anaerobic or waterlogged conditions (Lomax et al., 2011). However, different organic species such as methylarsenate [MAs(V)], dimethylarsenate [DMAs(V) or cacodylate] and trimethylarsine oxide [TMAsO (V)] of As have been also reported (Yin et al., 2011). Besides, use of organic As compound like ROX (4-hydroxyl-3-nitrophenylarsenic acid) and p ASA (4-aminophenylarsonic acid) in agricultural application poses toxicity and risk to human health (Zheng et al., 2014). Recent studies have shown that contamination of arsenic (As) in paddy soils is a widespread problem due to irrigation of As-laden groundwater and mining activities or uses of arsenical agrochemicals in other regions of the world (Williams et al., 2007; Zhu et al., 2008; Brammer, 2009). Although the main source of As exposures are drinking water and the food supply (Guha Mazumder et al., 2014), concern is growing over the human exposure of As through dietary consumption of rice (Meharg, 2004; Smith et al., 2009). Rice, being particularly efficient in assimilating arsenic from paddy soils becomes part of the food chain and develops arrays of diseases such as keratosis, cancer, cerebrovascular disease, diabetes mellitus, and kidney disease resulting into slow and painful death (Ma et al., 2008; Jomova et al., 2011; Rossman and Klein, 2011). In addition, the toxicity associated with organoarsenic compounds depends on associated organic functional groups and biological or environmental induced biotransformation (Zheng et al., 2014). The accumulation of As in rice may be attributed to two main factors: the reductive mobilization of As(III) in anaerobic paddy soils (Xu et al., 2008) and uptake of arsenite via the silicic acid pathway in rice (Ma et al., 2008). Plants exposed to As, develop reactive oxygen species (ROS) leading to photosynthetic pigment degradation, lipid peroxidation, electrolyte leakage and DNA damage (Rai et al., 2011). Malondialdehyde, a common oxidation product of polyunsaturated fatty acids, refers

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as TBARS has been considered as a marker of oxidative damage and commonly used to determine the stress level in the plants. To overcome the ROS toxicity, plants are equipped with various enzymatic and non-enzymatic antioxidant defense systems to reduce the stress level (Upadhyay et al., 2014). Thus, rice being an important dietary component for many people in the world, its quality needs to be assured.

Algae are an important component of aquatic environments and soil and play major role in bio-geochemical cycle (Ye et al., 2012). Recently, algae have received much attention due to their ability to absorption, sequestration and capacity to synthesize phytochelatins and metallothioneins that can form complexes of heavy metals and translocate them into vacuoles (Suresh and Ravishankar, 2004). Uptake of toxic elements by algae is basically dependent on the process of adsorption and metabolism-dependent active uptake (Lomax et al., 2011). Competence of green algae for metal accumulation is considered as a more attractive and potential technique for the restoration of metal contaminated water and soil. *Chlorella vulgaris* is widely distributed microalgae found mostly in fresh, stagnant and differently contaminated water and waste water, having a short life cycle and easier to handle in the laboratory (Rai et al., 2013). *Nannochloropsis* sp. is photoautotrophic, spherical, fast-growing and comparatively smaller than *C. vulgaris* (Sukenik et al., 2009; Kilian et al., 2011). However, to the extent of our knowledge, no work has been done about how algalization assists in As amelioration in rice. In this study, work is focused on comparative As tolerance and uptake in the rice plant inoculated with the *C. vulgaris* and *Nannochloropsis* sp. to assess their potential for amelioration of As toxicity.

2. Material and methods

2.1. Collection and cultivation of algae

Microalgae *C. vulgaris* and *Nannochloropsis* sp. were collected and isolated from As contaminated area of West Bengal, India and grown in the BG-11 medium. The medium consists of the following components; $NaNO_3$ (1.5 g/L), K_2HPO_4 (0.04 g/L), $MgSO_4 \cdot 7H_2O$ (0.075 g/L), $CaCl_2 \cdot 2H_2O$ (0.036 g/L), citric acid (0.01 g/L), ferric ammonium citrate (0.006 g/L), $Na_2 \cdot EDTA$ (0.001 g/L), Na_2CO_3 (0.02 mg/L) and trace metal solution. The trace metal solution contains H_3BO_3 (61.0 mg/L), $MnSO_4 \cdot H_2O$ (169.0 mg/L), $ZnSO_4 \cdot 7H_2O$ (287.0 mg/L), $CuSO_4 \cdot 5H_2O$ (2.5 mg/L), and $(NH_4)_6MoO_4 \cdot 4H_2O$ (12.5 mg/L).

2.2. Germination of rice seed

Rice (*Oryza sativa* L.) seedlings were obtained by growing rice seed var. triguna in a seed germinator. Rice seeds were washed with 70% ethanol for 30 s and sterilize in 2.5% sodium hypochlorite solution for 15 min. Then rinsed with Milli-Q water 5 times, and incubated for 2–3 d covered with soaked blotting paper in petridish.

2.3. Experimental setup

Rice seedlings (7 d old) approx. same size, weight and numbers (15–20) were transferred into a plastic tray containing 24 PVC cups for the anchoring and proper growth of plants in hydroponic condition and grown in 4 l modified Hewitt's nutrient medium (Liu et al., 2004). The pH of the media was maintained between 5.5 and 6.0 with the help of 0.1% KOH and HCl. After 7 d of growth and acclimatization, plants were inoculated with the thick culture (10%) of algae and left for 7 d for colonization, and then treated with 50 μM As(III) in a total 4 l nutrient solution. Rice grown in Hewitt nutrient medium served as control. The experiments were conducted in aseptic laboratory conditions. The harvesting of rice was done after 7 d of treatment. All the experiments were carried out under controlled laboratory conditions, i.e., 14 h light and 10 h dark, light intensity of approximately 280 μmol m^{-2} s^{-1}, 25 ± 2 °C temperature and 60% relative humidity in triplicate and repeated twice. Harvested rice plants were blotted to remove moisture content and measured the root, shoot length (cm) and fresh weight (g) with the help of metric scale and weighing balance.

2.4. Biochemical analysis

2.4.1. Photosynthetic pigments

The blotted dry rice leaves were crushed in 3 ml 80% acetone with the help of pestle and mortar under dark cold conditions and centrifuged at 8000*g* for 10 min. Supernatants were used for the estimation of chlorophyll content in treated and control plants following the method of Arnon (1949). Carotenoids content was calculated at the wavelength of 480 and 510 nm using the formula given by Duxbury and Yentsch (1956).

2.4.2. Protein content

Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin (Sigma) as standard. Plant material (100 mg) was crushed in 5 ml 10% chilled trichloroacetic acid (TCA) and centrifuged at 10,000*g* for 10 min. After decanting the supernatant, pellets were washed and resuspended in 5 ml of 0.1 N NaOH and again centrifuged at 10,000*g* for 10 min. Supernatant was mixed with alkaline Cu solution and Folin-phenol-ciocalteau reagent and leave for 30 min. The absorbance was recorded at 650 nm.

2.4.3. TBARS content

TBARS content was estimated according to the method of Heath and Packer (1968). 250 mg of plant leaves was crushed in 5 ml of 0.1% TCA and centrifuged at 10,000*g* for 10 min. 1 ml aliquot of the supernatant was mixed with 4 ml of 20% trichloroacetic acid (TCA) containing 0.5% of thiobarbituric acid and subjected to heat at 95 °C for 30 min followed by cooling in an ice bath and centrifuged at 10,000*g* for 10 min. The absorbance of the supernatant was taken at 532 nm and 600 nm using a spectrophotometer.

2.5. Antioxidant enzyme assay

2.5.1. Preparation of enzyme extract

Enzyme extracts were prepared by homogenizing 200 mg plant leaves in 2 ml of 100 mM potassium phosphate buffer (pH 7.5), containing 1 mM of EDTA and a pinch of polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 12,000*g* for 15 min at 4 °C. All the steps in the preparation of enzyme extract were carried out at 4 °C. The extract was used to measure the activities of antioxidant enzymes.

2.5.2. Superoxide dismutase assay

The activity of superoxide dismutase (SOD) was measured by the method of Nishikimi and Rao (1972), using the enzyme extracts. SOD activity was assayed by its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Inhibition of 50% shows the expression of 1 Unit (1 U) enzyme. A system devoid of enzyme served as control.

2.5.3. Ascorbate peroxidase assay

Ascorbate peroxidase (APX) activity was measured in the leaves of the rice plant by the method of Nakano and Asada (1981). The reaction mixture was prepared by adding 50 mM pentose