Bacteria mediated reclamation of Arsenic induced biochemical injuries in maize plant

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ABSTRACT

Arsenic is the ubiquitous toxic metalloid causing severe hazard in crop production. Contaminated groundwater used in irrigation causing increase soil Arsenic level and ultimately move in the food chain through crop in several parts of West Bengal. Present study was conducted for authenticating the potentiality of a naturally occurring arsenic tolerant bacteria for utilizing arsenic induced stress management in the maize cultivation. Here, chlorophyll a and chlorophyll b contents showed significant reduction on exposure of arsenic that can be attributed to the earlier report where heavy metal inhibits biosynthesis of chlorophyll and leading to senescence. Lipid peroxidation ability of the leaf tissues increased on the stressed plants even more than control in the bacteria treated experimental sets. Both the stress induced antioxidant defense system indicators of the plant tissue, i.e. peroxidase or enzymatic antioxidant and secondary metabolites (polyphenolic compounds) were higher in the arsenic treatment. As observed in the bacterial treatment set, those stress indicators were significantly lowered. Therefore it might be considered to explain the fact that application of bacteria might be able to accumulate the arsenic components from soil, thereby cleanse the environment for a stress-free growth of the potted maize plants. Thus, current study directed the application of A10 bacterial strain for possible protection of the experimented maize plant from the arsenic induced biochemical injuries.

Keyword: *antioxidant, arsenic, bacteria, biochemical, rice*

1. INTRODUCTION

Arsenic is the ubiquitous toxic metalloid causing severe health hazard of human being. Poisoning through arsenic, possess a major threat in heavily contaminated areas in different parts of India especially in West Bengal (Majumder et al., 2013). Significant increment in soil arsenic level in some part of West Bengal due to continuous use of arsenic contaminated groundwater for crop irrigations, has been reported (Sanyal and Dhillon, 2005) resulted into contamination of food chain and subsequently leading to deleterious of human health. The toxic metal, in agricultural field, is also absorbed by the plant which lead to impaired physiological condition and ultimately affects the crop production. Therefore, to adopt a low cost strategy to overcome this environmental hazard is a front-line concern to the scientific community.

Few microorganisms in nature, can tolerate the high arsenic toxicity and recovers the soil health by various modes like precipitation, chelation, compartmentalization, extrusion and biochemical transformation (Ghosh et al., 2018). In recent years, various arsenic tolerant bacterial strains has been utilized to such bioremediation program. Present study was conducted to check the potentiality of a naturally isolated arsenic tolerant bacteria to assist arsenic induced stress management in the maize plant.

2. MATERIAL AND METHOD

2.1 Plant material and treatments

Pot experiment was conducted to evaluate the effects of selected arsenic resistant bacteria (A10) on arsenic treated maize seedlings. For this experiment, soil was collected randomly from the university campus at Kalyani. Before starting the experiment soil and sand were sterilized in autoclave (15 lb pressure for 1hour for 3 consecutive days). Every pot was filled one third by sand and the upper remaining portion filled with sterilized soil. Maize seeds were surface sterilized by soaking in 0.1% mercuric chloride solution for 5 minutes followed by rinsing with sterile

distilled water and then imbibed in selected bacterial broth (A10) for 30 minutes. A bacterial strain having arsenic resistance potentiality was isolated, named A10, from arsenic contaminated ground water of Chakamdanga block of Chakdaha, Nadia, West Bengal, India which was previously reported as arsenic prone area. The strain was used in experimental set up for inoculating maize seedling (cultivar: S-35). After imbibition the seeds were sown in three different sets of pots each with three replicates, namely control, stress (5µM sodium arsenate irrigation) and stress + bacteria (A10) (Figure 1).

2.2 **Estimation of Lipid Peroxidation**

Lipid peroxidation was assayed by quantification of malondialdehyde (MDA) following the method of Strasser et al. (1995) with some modifications. In brief 100mg leaf sample was homogenized and extracted in 20ml of 0.1% TCA solution and then centrifuged at 12000g for 10 min at 4˚C. 1ml supernatant was taken and added 4ml of 20% TCA containing 0.5% TBA to it and subjected for 30 min incubation at 95˚C. At the end of incubation period the reaction mixture was placed on ice bath and centrifuged at 12000g for 10min. The absorbance of supernatant was recorded at 532nm and the value for non-specific absorption at 600nm was subtracted. MDA concentration was detected using the extinction coefficient of 155 mM^{-1} cm^{-1} following the formula:

$$\text{MDA content (nmol)} = \Delta\text{Abs (532-600) nm} / 1.55\times 10^5$$

2.3 Estimation of leaf chlorophyll and carotenoid contents

The chlorophyll and carotenoid contents were determined by the methods described by Arnon et al. (1954). Leaf tissue from each sets were homogenized in chilled acetone and centrifuged. The absorbance of supernatants were spectrophotometrically measured at 480, 510, 645 and 663 nm for Chl a, Chl b, total chlorophyll and carotenoids, respectively.

2.4 Detection of Total Phenol

Total Phenol content in the samples was detected by using Folin-Cio-calteu reagent following the method of Singleton and Rossi (1965) with some modifications. Briefly the plant extract (0.5mg/ml) was taken and mixed with 2ml FC reagent (diluted previously 10 times) and 1.6ml Sodium carbonate (7.5%).Incubated for 30 minutes at room temperature and then the absorbance was measured spectrophotometrically at 765nm. Here Gallic acid was used to prepare the reference curve for determining equivalent concentration of corresponding compound.

2.5 Estimation of peroxidase enzyme activity

A total 150mg of fresh leaf tissue was taken separately from three sets and homogenized in chilled mortar pestle with 5 ml of enzyme extraction buffer containing 0.1M phosphate buffer, pH 7.0; 0.25 mM ethylene diamine tetra-acetic acid (EDTA); 2.5 mM cysteine HCl and 2.5% polyvinyl pyrollidone (PVP). Then the extract was centrifuged at 10,000 g at 4°C for 15 min. The supernatant was used for the estimation of peroxidase activity. This activity was estimated according to the method of Panda et.al (2003) with some modifications. 1ml (0.1M, pH-7.0) potassium phosphate buffer was taken in a test tube and 20µl guaiacol solution was added followed by 40µl of enzyme extract. Before starting assay the temperature was maintained at 25˚C. Reaction was started after the addition of 15 µl H_2O. After mixed it well, absorbance was taken at 436nm in spectrophotometer.

3. RESULT AND DISCUSSION

Photosynthetic pigments such as chlorophyll-a, chlorophyll-b and carotenoids content reduced when the plants grown under arsenic stress. However the pigments compositions were elevated significantly when applying the test bacteria. Qian et al. (2009) reported that, heavy metal contamination has inhibitory effect on enzymes involved in pigment biosynthesis that results declining chlorophyll and carotenoid contents. Muradoglu et al. (2015) stated that, chlorophyll pigments are one of the main reasons of heavy-metal injury in plants. Here, chlorophyll a and chlorophyll b contents showed significant reduction on exposure of arsenic (Figure 1) that can be attributed to the earlier report where heavy metal inhibits biosynthesis of chlorophyll and causes senescence (Fang et al., 1998).

The increased lipid peroxidation activities are indication of higher production of reactive oxygen species than normal condition. Plants growing in stressful environments, generate excess free-radicals which is accumulate in the cells (Muradoglu et al., 2015). These free radicals leads to lipid peroxidation of bio-membranes, the end product of the reaction is MDA. So, the MDA content is considered as an indicator of physiological stresses and the aging

process (Chen et al., 2003). Lipid peroxidation ability of the leaf tissues increased on the stress exposure that is reduced even more than control in the bacteria treated sets (Figure 1). Therefore, bacterial treatment seems to have positive effect on reducing the arsenic induced stress injury of the maize plant and maintain the normal homeostasis of the said plant.

Overproduction of toxic free radicals production in plants is maintained by antioxidative enzymes which hunt excessive free radicals and protect the cells from oxidative damage (Duquesnoy et al., 2010). Here, both the stress induced antioxidant defense system indicators of the plant tissue, i.e. peroxidase or enzymatic antioxidant and phenolic.

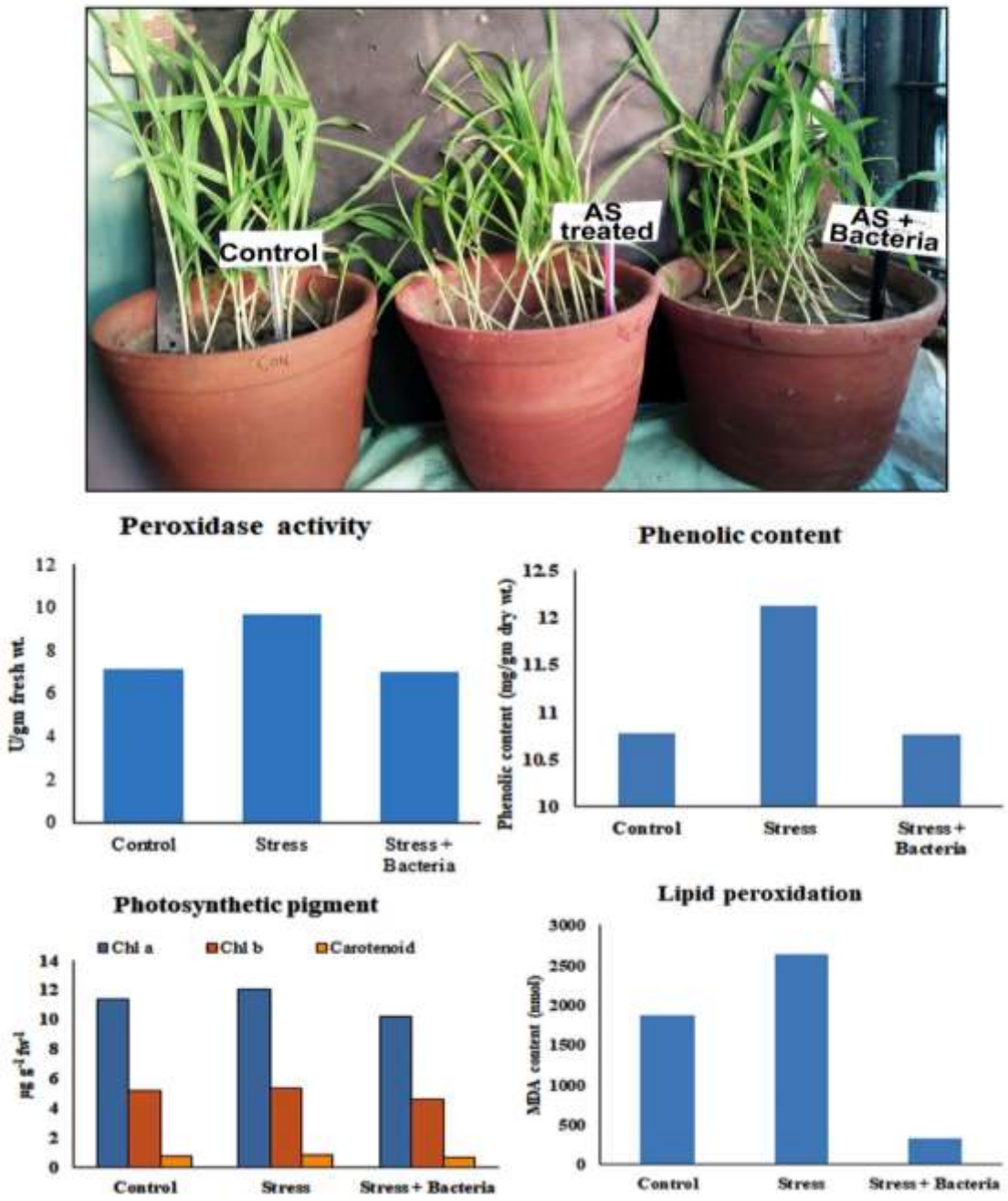

Figure 1: Arsenic and bacteria treated maize plants of 21 days maturity and occurrence of various biochemical parameters

compounds or the secondary metabolite class of antioxidants, were higher in the arsenic treatment. As observed in the bacterial treatment set, those stress indicators were significantly lowered (Figure 1). Therefore it might be considered to explain the fact that application of bacteria might be able to accumulate the arsenic components from soil, thereby cleanse the environment for a stress-free growth of the potted maize plants. Thus, current study directed the application of A10 bacterial strain for possible protection of the experimented maize plant from the arsenic induced biochemical injuries.

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