Changes in growth responses in rice plants grown in the arsenic affected area: implication of As resistant microbes in mineral content and translocation

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Abstract

Arsenic (As) is widely distributed naturally as well as an anthropogenic occurring toxic metalloid. Its unique interaction with the ecosystem has led to a broad study into its physico-chemical properties and lethal behavior in the environment. This study deals with the mineral nutrient profile in the *Oryza sativa* (rice grains) with various doses of As and bacterial strains. Results revealed that rice supplemented with As resistant bacteria BBAU/MMM₁ (*Bacillus litoralis*) and BBAU/LP₃ (*Bacillus infantis*) exhibited enhanced growth characteristics by 32.0% and > 23.7% respectively with respect to control. Arsenite [As(III)] treated rice supplemented with different bacterial strains represents a significant positive response in the order of 10–17%. Further, the mineral nutrient content in As(III) (100 µM) treated rice grain was decreased by Fe (34.23%), Zn (32.22%), Mn (17.3%), Cu (25.34%), Co (29.77%) and Se (50.62%), respectively. However, bacterial inoculated rice plant exhibited Fe (7.34%), Zn (65.02%), Mn (14.54%), Cu (22.04%), Co (46.37%), and Se (203.47%), increased mineral content in the case of *B. litoralis* in comparison to rice treated with As(III). Results emphasize that bacterial strain, *B. litoralis* could reduce the toxic impact of rice induced by As and enhance mineral nutrient content in rice.

Keywords Arsenic · Metal resistance · Mineral content · *Oryza sativa* · Translocation

1 Introduction

Arsenic (As) is a ubiquitous and carcinogenic in nature that has raised many serious concerns worldwide. A major source of As contamination in soil is groundwater such as mining, volcanic activities, fossil fuel burning, fertilizers and also the use of pesticides [32, 38]. In recent years, As poisoning grasp millions of peoples due to the use of As contamination in drinking water and crop plants. Rice being the major staple crop and the way of its production i.e., flooded environment, accumulate an efficient amount of As, which further exposes to human beings through food chain contamination [40]. Rice is grown in flooded soil, accumulated more As increasing high concentration in the grain because of the release of by As reducing bacteria and reductive dissolution of

iron hydroxides in anaerobic condition [13]. In addition to the human being, As limits the accumulation of other important mineral nutrients in the grain resulting in the production of rice with a low level of mineral content [2, 3]. Rice is a vital contributor of energy (70%) and protein (50%) over half of the world [14], it is imperative to study the interaction and correlation of different minerals with As in rice plants. Microbes mediated As biotransformation may participate a vital function in the fate and toxicity of As [39]. Different type of anaerobic and aerobic bacteria present in paddy field contaminated with As significantly reduces the toxic impact of As. Arsenic mainly exit in trivalent (As(III)) form in flood condition which is more toxic than pentavalent form As(V) [4, 32, 36]. The bacteria present in soil solution metabolizes As(III) to As(V) through the process of methylation. Oxidation

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of As(III) to As(V) is work as a detoxification mechanism operated by the microorganism [5, 25]. Mukhopadhyay et al. [22] have reported that the biogeochemical cycle of As depends on microbial mediated oxidation, reduction and methylation processes which influence the mobility and distribution of As species in the environment. In this way, microbes also control the stress-induced oxidative damage response, growth, biomass and mineral content in paddy plant grown in As contaminated soil. Arsenic influences the uptake of important mineral nutrients in plants by minimizing transportation, interaction with other metals, competition, root cell degradation and reducing metabolic processes [3, 6, 30]. The synergistic, additive and antagonistic relationship between different metal alters their bioavailability in the solution [24]. Decreased mineral content is also caused by ion leakage from damaged root and immobilization of elements in root resulting in a strong deficiency in the shoot. A number of the authors have been reported the lower translocation of mineral under heavy metal stress in plants [1, 3, 6, 7, 9, 17, 29, 37]. However, no study is still done on the effect of As and bacterial strain in the mineral acquisition, translocation, and growth.

In the present study, we are trying to find out the impact and interaction of As and arsenic-resistant bacteria singly and in combination on translocation and accumulation of mineral nutrient and biogeochemistry of As. Further, it was also prospecting in term of growth, toxicity and yield of the crop plants.

2 Materials and methods

2.1 Isolation and screening of As resistance bacteria

Soils samples were collected from different sites of Bahraich viz, Mari Mata Mandir (Block-Mihinpurwa), Govindpur (Block-Payagpur), Rashulpur (Block-Phakharpur), Lalpur (Block-Payagpur and Kochwa (Block-Rupai Deeh). Samples were collected in bio-hazardous poly bags, stored at 4 °C. 1 g soil samples were dissolved in 1 ml distilled water for the isolation of bacteria. 100 µl of the sample was taken in the Eppendorf tube containing 900 µl of distilled water. Before further samples analysis shakes well the Eppendorf tube having 10^{-1} dilution. Similarly, 2–5 dilutions, 25 µl of the sample were spread on pre-sterilized Petri dish. Bacterial colonies appeared after 24–48 h on agar plates. The isolated strains were cultured in nutrient broth media. For the analysis of As

resistance, isolated bacteria were subjected to treatment by different concentrations of As(V) ($100\text{--}600\text{ mg l}^{-1}$) for 24 h at 37 °C incubation.

2.2 Germination of rice seedlings

In this study, used rice grains were purchased from Surya product Pvt. Ltd. And further, it was sterilized with HgCl_2 (0.1%) solution for 1 min, washed with deionized water and soak in distilled water for 24 h. After that, these seeds were spread in pre-sterilized soaked blotting paper in a tray. The seed was germinated in a seed germination chamber at a constant temperature, light, and humidity (16-h light period with a light intensity of $350\text{ mmol m}^{-2}\text{ s}^{-1}$; 25/20 °C day/night temperatures; and 60% relative humidity). Finally, by using Hewitt nutrient media [20] rice seedlings were kept in light at a controlled condition for growth (10 days).

2.3 Experimental design

In controlled environmental parameters viz. light, temperature and humidity the pot experiment were performed. Pre-sterilized soil was used for filling the pot, which ensures microbes free experiments. In this study, two concentration were used i.e., 50 µM As(III) as a low dose and 100 µM As(III) as high dose were applied in rice plant. 100 µl bacterial inoculums were prepared in distilled water. The bacterial inoculums were applied in As(III) treated and control rice plant. The mass culture of isolated bacterial strain was prepared in Erlenmeyer flask containing 300 ml nutrient media. The mass cultures were incubated at 37 °C at 2–4 g in an orbital shaker. After the growth pellet was prepared by centrifuging the culture at 10,000 rpm for 20 min. Bacterial inoculums were prepared by dissolving collected biomass in sterile distilled water. All the experiments were performed in pots for 100 days and in three replicates.

2.4 Harvesting of rice

In this study, selected rice plants were grown to grain level. The plants were harvested after 100 d of maturity. The grown plant was uprooted safely from the pot, further roots were washed thoroughly with tap water. Rice plants were chopped into the root, shoot and grains for further study.

2.5 Biochemical analysis of rice

2.5.1 Estimation of growth characteristics

Growth parameters in the form of root, shoot length (cm) and fresh weights (g) were measured by a metric scale and weighing balance respectively.

2.5.2 Estimation of mineral content in rice and soil

Jackson [12], methods (stannous chloride) was used for the estimation of phosphorous content in soil and plant samples. Whereas other metal content like Fe, Zn, Mn Cu, Co, and Se rice grains was estimated by acid digestion, in which oven-dried sample (0.5 g) were transferred in HNO₃ (3 ml) for the digestion. Simultaneously, for the soil metal analysis, samples were dried, grind and sieve (< 2 mm) than 0.2 g soil was digested in HNO₃: HF (1:1) at 120 °C for 2 h and 140 °C for 4 h [21], filtered in Milli-Q water (10 ml) and stored at 4 °C. Further, for the analysis of arsenic content harvested plant samples were dried till the constant weight at 70 °C for 24 h. 10 mg samples (root and shoot) were digested at 80 °C in HNO₃ and HClO₄ solution (3:1 v/v). Finally, samples were allowed to dissolve and cool in HNO₃ (0.6%). All these prepared samples analyses were done with the help of Atomic Absorption Spectrophotometer (AAS).

2.6 Statistical analysis

In this study, all the determinations were carried out in three replicates ($n=3, \pm$ SD). Analysis of variance (ANOVA), Tukey's test analysis was performed to determine the significant difference between treatments by using SPSS 17.0 software.

3 Results and discussion

3.1 Isolation and screening of As resistance bacteria

Bacterial strains were collected and isolated from agriculture field and named as BBAU/MMM BBAU/GP, BBAU/RP, BBAU/LP, and BBAU/KC on the basis of the sampling site. After the isolation, bacterial strains were screened for the As resistance. Six concentration of As(V) i.e., 100, 200, 300, 400, 500 and 600 mg l⁻¹ were used for screening of As resistance bacteria. Of all the isolated strain from different sampling site, strain BBAU/MMM₁ (*Bacillus litoralis*) and BBAU/LP₃ (*Bacillus infantis*) were found to be more effective resistant as evidenced by positive growth at the higher concentration of As(V) (600 mg l⁻¹), while other strains showed toxic effect.

3.2 Growth characteristics of rice

Rice plants supplemented with As resistant bacterial strains showed positive growth in the form of root length, shoot length and biomass with respect to control (Fig. 1). The percentage increased in root length shoot length and biomass were in the order of 32%, 22.14%, and 61.40% respectively with BBAU/MMM₁ *B. litoralis* strain. In the case of rice inoculated with strain BBAU/LP₃ *Bacillus infantis*) a similar trend was observed which was 23.77%, 16.84%, and 48.15% respectively. A concentration-dependent significant reduction in growth was observed in rice treated with low (50 µM) and high dose (100 µM) of As(III). However, in a combination of bacteria and As(III), the plant showed a positive response, which was more pronounced in the case of As treated rice supplemented with bacteria strain BBAU/MMM₁ *B. litoralis*. Results demarcated that As resistant bacteria play a significant role in the plant to overcome the toxic impact of As.

3.3 Mineral elemental accumulation

The accumulation impact of different metals in rice treated with different concentration of As(III) and microbes has been depicted in Fig. 2. Results showed that all the element (Fe, Zn, Mn, Cu, Co, and Se) content were decreased under As(III) stress according to the concentration gradient. Similar to this, increasing trends were also observed in all the mineral content in rice inoculated with bacteria which was maximum with bacterial strain BBAU/MMM₁, except in the case of Se and Zn. Maximum reduction was observed with Se(50.62%) while minimum with Mn (17.3%). Results revealed that rice plant treated with low dose of As(III) supplemented with bacterial strain exhibited a positive response in the form of increased metal content in comparison to rice treated with As(III) only.

3.4 Arsenic accumulation

Rice plant treated with a different combination of As(III) and bacteria accumulated the different level of accumulation (Table 1). Results showed that accumulation of total As was significant increased with increased concentration by twofold as compared to low dose (50 µM). In the case of rice treated with bacterial strain, BBAU/LP₃ decreased accumulation by 32.84% and 40.9% at a low and high dose of As(III) respectively as compared to their As(III) control. On the other hand, reduced was further increased up to 51% in the As-treated rice supplemented with bacterial strain BBAU/MMM₁. Overall results suggested that microbes play a significant role and minimizing the

Fig. 1 Growth characteristics (root shoot length and fresh weight) of rice plants treated with different concentration of As(III) and bacteria. All the values are means of 3 replicate ($n=3$) $\pm$ S.D. ANOVA

significant at $p < 0.01$. Different letters indicate significantly different values between treatments (Tukey's test, $p < 0.05$) (C control; B 1 BBAU/MMM₁, B 2 BBAU/LP₃, L low, H high, As arsenite)

accumulation and detoxification in plants. Besides, strain BBAU/MMM₁ (*Bacillus litoralis*) showed a strong potent role in As bioremediation.

3.5 Discussion

Microbes play an important role in metal translocation and bioavailability through the process of biotransformation, oxidation, precipitation, and sequestration. Huang et al. [10] also reported that microbe reduces the As accumulation by changing soil pH, redox balance and precipitation, etc. During the biogeochemical cycle of As a huge number of microbes are involved. There are various types of mechanism are occurred such as redox reaction, methylation, precipitation, biosorption by cell biomass, entrapments

through cellular capsule and production of induced proteins, active cell transport, in the bacteria, fungi, algae, mosses, higher plants and macrophytes for the removal or mitigation of heavy metals including As [7, 23, 27, 35, 38].

The increase of concentration of As in the different plant was very high in aerobic and anaerobic condition, which finally affect the yield of rice parameters and significantly reduces the yield. In anaerobic condition soil released iron oxides which may also increase the inorganic arsenic content [28, 32]. It is a well-known fact of decreased growth characteristics of the plant under As stress reported by many authors [6, 18, 28, 38, 40]. This might be due to As-induced toxicity which leads to degeneration of root cell, membrane permeability, chloroplast and pigment denaturation degradation

Fig. 2 Accumulation of different mineral nutrients (Fe, Se, Mn, Zn, Co and Cu) in rice grain treated with different concentration of As(III) and bacteria. All the values are means of 3 replicate ($n=3$) $\pm$ S.D. ANOVA significant at $p < 0.01$. Different letters indicate

significantly different values between treatments (Tukey's test, $p < 0.05$) (C control, B 1 BBAU/MMM₁, B 2 BBAU/LP₃, L low, H high, As arsenite)

[16]. Growth recovery in rice inoculated with As and bacteria is the results of utilization of different mineral nutrients and increased availability to the plants

through the process of biotransformation, mobilization, organic matter breakdown and solubilization of important nutrients such as phosphorus and nitrogen

Table 1 Arsenic accumulation in rice grains treated with different concentration of As(III) and bacterial strain

Treatments	Arsenic accumulation (mg kg ⁻¹ dw)
C	–
B 1	–
B 2	–
As-L	85.80 ^{bc} ± 7.34
As-H	176.60 ^d ± 17.18
B 1 + As-L	57.62 ^{ab} ± 4.68
B 1 + As-H	104.20 ^c ± 15.37
B 2 + As-L	52.76 ^a ± 9.98
B 2 + As-H	85.80 ^{bc} ± 8.77

C control, B 1 BBAU/MMM₁, B 2=BBAU/LP₃, L low, H high, As arsenite

All the values are means of 3 replicate (n=3) ± S.D. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values between treatments (Tukey's test, $p < 0.05$)

[31]. Bacterial-mediated increased growth response in different plants was also reported by the various author [19, 26, 34]. In the case of rice supplemented with As resistant microbes and As, respond positive impact due to the uptake of As by microbes, reduced translocation and thus induced tolerance responses in the form of increased growth characteristics [31].

In the flooding environment, iron reducing bacteria are in huge amount, which reduces Fe-oxyhydroxide and increase the As solubility in soil [8]. It also stimulates the transformation of AsV–AsIII and methylated As species. Somenahally et al. [33] found that in both intermittent and continuous flooding showed the total As concentration in rhizospheric soils and grains were considerably reduces in intermittent flooding environment than the continuous flooding. Rice is the principal source of important minerals for human health and fulfills the mineral requirement of more than half of the world [11, 15]. Reduction in metal uptake by the rice plant is a well-known effect of As-induced toxicity. Iron is frequently occurred macronutrients in paddy field and has a strong effect on As dynamics and bioavailability through the process of co-precipitation, adsorption and sequestration of As in iron plaque [2]. So far there is a number of biological as well as physico-chemical methods are available, unfortunately, these are not much more effective for the removal of toxic metalloid arsenic. In the present study, isolated bacteria may be useful for the better growth of crops with high arsenic resistance capacity.

4 Conclusion

Due to uncontrolled industrialization and urbanization discharge wastes contains a huge amount of toxic heavy metals contamination, which has high deleterious effects on human, animals as well as plants. An agricultural soil sample which was collected from sampling sites, contaminated with toxic metalloid As, which mainly affects agricultural crops. The bacterial culture isolated from As contaminated sites found highly As resistance and also helpful in enhancing crop growth and another development process. As resistant microbes inoculated in rice plant enhances the growth characteristics in the form of root-shoot length and biomass. Further, mineral nutrient content in rice grain under As with bacteria BBAU/MMM₁ *B. litoralis* significantly increased the content. Thus, the bacterial strain could be employed in the As contaminated area for better growth and nutrient status of the crop plants.

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Compliance with ethical standards

Conflict of interest Corresponding author and the co-author of the manuscript have no conflict of interest pertaining to this work.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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