

**Implications and prospects of microbial based
integrated management of arsenic remediation in
rice (*Oryza sativa* L.)**

SUMMARY

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5. SUMMARY AND CONCLUSION

Arsenic (As) is naturally occurring well known deadlier non threshold carcinogen of natural and anthropogenic origin. Rice (*Oryza sativa* L.) is a staple diet for more than half of the world's population. Rice is particularly efficient in accumulating As and paddy fields in South and South-East Asia, which are highly contaminated with As. Particularly, in West Bengal, India, where millions of people depend on rice as subsistence diet are getting affected. It is also a threat to sustainable agriculture in affected areas.

The main objective of this study was rhizospheric microbes grown in As contaminated soil of West Bengal, India were isolated and characterized for their As resistant potential and bioremediation potential. The work entailed studying the effects of As on plant metabolism, uptake and toxicity along with the understading the role of antioxidant and thiol metabolism in alleviation of deleterious effects of As in rice plants. Further, the study evaluated the potential of arsenic resistant bacteria supplementation on lowering the As level in rice plants.

Physicochemical analysis of soil sample collected from different As contaminated sites of West Bengal, India showed that out of three selected sites, The water holding capacity (WHC) was high in the Chinsurah site. In the case of inorganic nutrients such as N and P, it was maximum found in the site Chinsurah site . The maximum contamination of As in all the studied site were reported in site Biranagar site which was 17.18 mg/kg.

The species of As significantly reduced the root length; the maximum being during AsIII exposure in IET-19226. Shoot length of BRG-12 and IET-19226 increased at lower concentration of AsIII and AsV followed by a decline at higher doses.. Photosynthetic pigments showed significant decline in chlorophyll *a*, *b* and chlorophyll total at As exposures with more effect being in BRG-12 in comparison to IET-19226. Carotenoid content showed increase at low concentrations but decrease in higher concentration in IET-19226. The maximum induction in protein content was observed in IET-19226 at equimolar (10 μ M) concentration of AsV and AsIII, however, maximum decline in protein level was observed in BRG-12 at higher exposure.

Rice plants treated with different dose of As and bacterial inoculants showed alteration in growth. Shoot length approx. 50% reduction was observed at high dose of As(III). In the case of rice plant treated with the combination of bacterial strain and As improvement in root shoot length was observed in comparison to rice treated with As alone. However, no significant improvement root length was observed under bacterial inoculated with different indication of dose of As. Reduction in root and shoot length is clear cut indication of As induced toxicity. It may be due to As decrease the uptake and translocation of other micro element and nutrients by adsorption on root cell, cell membrane damage and reduced availability of nutrient required for the growth of the plants.

Arsenic induced reduction and destruction of chlorophyll machinery has been observed in rice plant treated with low and high dose of As(III) which was more prominent with high concentration of As(III). However, increased chl *a*, chl

b and total chl content was observed in shoot inoculated with bacterial strain as compared to control.

Rice plant treated with different dose of As(III) showed that rice root accumulated high content of As than shoot which increased with increasing the concentration of As(III). Results showed that rice root accumulated 1508 $\mu\text{g}/\text{kg dw}$ As and 2578 $\mu\text{g}/\text{kg dw}$ at 5 and 10 ppm, respectively. In the case of shoot it was 400 and 688 $\mu\text{g}/\text{kg dw}$, respectively in comparison to control. Arsenic treated rice supplemented with bacterial inoculants showed approx. 50% reduction in accumulation which was 738 and 1385 $\mu\text{g}/\text{kg dw}$, respectively, as compared to their respective control. The accumulation of As in husk and grain was 166 and 95.06 $\mu\text{g}/\text{kg dw}$ at low dose of As(III). In the case of rice plant treated with combination of bacterial and high dose of As(III), 37.92% reduction was observed in grain while in husk it was 40.97% as compared to the rice treated with As(III) alone.

The concentration of all the studied element (Fe, P, Zn, Mn, Cu, Co, and Se) decreased under As(III) stress. However, increasing trends were observed in the mineral content in rice grain inoculated with bacteria. Maximum reduction was observed in Se (39.25%) while minimum in Cu (16.62%) in rice plant treated with high dose of As(III) as compared to control. In the case of rice grain inoculated with bacterial strain maximum increased was observed in Co(40.47%) followed by Cu (34.88%), Mn(30.41%), P(30.01%), Fe (26.94%), Zn(22.31%) and Se(16.51%) as compared to control. Further, rice plant treated with the combination of As and bacterial inoculants showed decreasing trend as compared

to their As(III) control. Maximum reduction was observed in Zn (52.5%) in rice grain cosupplemented with high dose of As(III)+bacteria and minimum with Mn(7.44%) Results revealed that rice plant treated with As(III) (5 and 10) supplemented with bacterial strain showed positive response in the form of increased mineral content.

Rice plant treated with different concentration of As and bacterial strain showed cellular toxicity by increasing the MDA content which was significantly increased in the case of high dose of As as compared to control. It was observed that rice treated with high dose of As(III) and bacteria separately showed 30.9% increased in MDA level while no significant change observed with rice inoculated with bacteria only. In the case of rice plant root and shoot treated with combination of low dose of As(III) and bacterial inoculants, MDA content decreased by 12.05% and 30.34% respectively while with high dose of As(III)+bacterial inoculants it showed 14.2% and 20% as compared to their As(III) control.

H₂O₂ content in rice root and shoot treated with high As(III) showed increased level (0.592 and 0.289 mmol/g fw) in comparison to control (0.300 and 0.169 mmol/g fw). In the case of rice treated with low dose of As(III), no significant change was observed. H₂O₂ content in Rice plant treated with bacterial inoculants increased in the root while decreased in shoot as compared to control. The co-application of bacterial strain and different doses of As, recovered the level with respect to rice treated with As alone. Overall results showed that inoculated bacteria reduced the toxic level of As by decreasing the H₂O₂ content

in rice treated with low dose of As and Bacterial inoculants (0.342 mmol/g fw) in comparison to As control (0.592 mmol/g fw).

Thiolic legends presented in Cys, GSH and GSSG in figure 2 (A-D). Cysteine content in rice root and shoot treated with high dose of As increased (107.26 and 191.25 nmol/g fw) in comparison to control root and shoot (69.20 and 128.8 nmol/g fw). In the case of rice plant treated with bacterial inoculants also increased cysteine content in root and shoot. However, no significant change was observed in the case of root. Rice plant treated with combination of bacterial inoculants and low dose As(III) showed decreased cysteine content in root in comparison to As while increased in shoot. In the case of high dose +bacterial inoculants cysteine content increased to 131.08 and 254.95 nmol/g fw respectively in root and shoot in comparison to As(III) alone. Cysteine is important amino acids and acts as antioxidants inside plant cell.

The glutathione (GSH) content in rice root treated with high dose of As(III) increased by 32.5% while no significant change was observed under low As(III). In the case of rice shoot treated with high dose of As(III) showed increased GSH value from 67.29- 69.20 $\mu\text{mol/g fw}$ and with low As(III), GSH content was decreased from 67.29-59.50 $\mu\text{mol/g fw}$ in comparison to control. Rice plant treated with bacterial inoculants singly showed increased GSH content in comparison to control. In the case of rice plant treated with the combination of As(III) and bacterial inoculants, showed reduced GSH content in comparison to As(III) alone. The oxidized glutathione (GSSG) content in rice root treated with

different combination of As and bacterial inoculants either singly or in combination showed similar trends.

Superoxide dismutase activity in rice plant treated with singly and combination of As(III) and bacterial inoculants showed increased response which was more in the case of rice treated with high dose of As(III) separately. The SOD activity was increased by 13.2% in root treated with bacterial inoculants. In the case of high dose of As(III), the SOD activity in root and shoot was increased by 52.5% and 16.12% as compared to control. Rice plant treated with combination of As(III)+ bacterial inoculants showed maximum reduction in SOD activity in root (128.16 U/ mg protein) as well as shoot (204.33 U/ mg protein) under low dose of As(III) as compared to As(III) only.

The catalase activity was increased with increased concentration of As(III) in rice root (7.33 Unit/ mg protein) as compared to control (4.73 Unit/mg/ ptotein). A marked increased (21.7% and 24.1%) in catalase activity was also observed in rice root and shoot inoculated with bacteria. In the case of rice root and shoot treated with the combination of As(III)+bacterial inoculants, reduction in catalase activity was observed in comparison to rice treated with As(III) alone.

Rice plants treated with bacterial inoculants and As(III) separately and in combination showed alteration in GR activity. In the case of rice root treated with high dose of As(III), GR activity increased from 0.34-0.67 while with low As(III), it was 0.58-0.84. Rice treated with high dose of As(III)+ bacterial inoculants, showed reduction in activity in root and shoot by 14.9% and 26.19% respectively. Rice plant treated with the combination of As(III) and bacterial inoculants showed

reduced GR activity in rice root and shoot in comparison to As(III) control reflecting bacterial induced tolerance responses.

The present study evaluated the growth, mineral content and biochemical effects of arsenic exposures with inoculation of arsenic resistant bacteria in rice plant. The results of the study the following concluding remarks:

- In the present study, rhizospheric microbes grown in As contaminated soil of West Bengal, India were isolated and characterized for their As resistant potential and bioremediation potential.
- Physicochemical analysis of soil sample collected from different As contaminated sites of West Bengal, India showed that out of three selected sites, maximum pH was found in the site purbosthali with high level of electrical conductivity (159.7 $\mu\text{s}/\text{cm}$), porosity (99.42 %) and total organic carbon (1.56 %) as compared to other sites.
- The water holding capacity (WHC) was high in the site chinsurah whereas minimum WHC was recorded in site birnagar.
- The maximum contamination of As in all the studied site were reported in site birnagar which was 17.18 mg/kg.
- Out of three selected bacteria from different site, bacterial isolates (BBAU/CH) showed good growth, high tolerance and resistant to the level of 100 mM of As(III).
- Arsenic treated rice plant inoculated with isolated bacterial strain (BBAU/CH) showed increased growth of the rice plant in term of root length, shoot length, fresh weight and chlorophyll content.

- Reduced translocation and accumulation root (2 fold) and shoot (1.5 fold) of As in rice inoculated with bacterial strain was observed.
- Rice inoculated with bacterial strain exhibited reduced lipid peroxidation, H₂O₂ production, induces antioxidants (cysteine, GSH, and GSSG) and enzymatic activities (SOD, CAT, and GR) were observed in rice root and shoot.
- The mineral status in grain of As(III) (high dose) treated rice exhibited alteration in mineral content and showed maximum reduction was observed in Se (39.25%) while minimum in Cu (16.62%).
- Bacterial strain (BBAU/CH) may be used as potent bioremediator for rice grown in As contaminated land or area.

Further, financial support and opportunity is needed for the detailed study and research.