AMBUJ BHUSHAN JHA

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Qualification/Educational Background

Present status	Working as a **Research Associate** at Institute of Genomics and Integrative Biology (IGIB), Council of Scientific & Industrial Research, Delhi, India
1999-2003	**PhD** on the topic "*Effect of arsenic toxicity on nitrogen assimilation, sugar metabolism and antioxidant system in rice plants*" from BHU, India
1996-1998	**Master of Science** in *Biochemistry*, BHU
1993-1996	**Bachelor of Science (Hons.)** (*Botany, Chemistry, Zoology*), BHU

Professional Experience

Research Associate	***Project title: "***Characterization, expression and regulation of genes encoding nitrate reductase under stresses in rice plants." Institute of Genomics and Integrative Biology (IGIB), Council of Scientific & Industrial Research, Delhi, India (Research adviser: Dr. Hasi Das)
Post Doctoral Research	***Project title:*** "Enzyme purification, characterization and expression of enzymes of antioxidative defense system under arsenic toxicity." Department of Biochemistry, Banaras Hindu University, Varanasi, India (Research adviser: Prof. R. S. Dubey) ***Project title:*** Gene identification of mutant obtained by Tn5 mutagenesis of *Rhizobia* Institute of Genomics and Integrative Biology (IGIB), Council of Scientific & Industrial Research, Delhi, India (Research adviser: Dr. Hasi Das)
Doctoral Research	***Research topic:*** "Effect of arsenic toxicity on nitrogen assimilation, sugar metabolism and antioxidant system in rice plants" Department of Biochemistry, Banaras Hindu University, Varanasi, India (Research adviser: Prof. R. S. Dubey).
Pre-Doctoral Research	***Project Title:*** "Purification of calmodulin from filarial parasite *Setaria cervi*." Summer training at the Division of Biochemistry, Central Drug Research Institute, Lucknow, India (Research adviser: Dr. J. K. Saxena).

Technical Expertise

Biochemistry	Colorimetric and spectrophotometric estimation of protein, enzymes; enzyme kinetics; 1-D (Native, SDS) and 2-D polyacrylamide gel electrophoresis (PAGE); isoelectric focusing
Protein Purification	Acetone fractionation, ammonium sulfate precipitation; dialysis; ion exchange chromatography, gel filtration chromatography
Immunology	Enzyme linked immuno sorbent assay (ELISA); western blotting
Microbiology	Light Microscopy; preparation of culture media; growth and maintenance of rhizobial culture; bacterial staining
Molecular Biology	Isolation of plasmid and genomic DNA; purification of isolated genomic DNA; polymerase Chain Reaction (PCR) analysis; agarose gel electrophoresis

Awards/Scholarship

Year 2003	***Awarded ISCA Best Poster Presentation Award*** in the ***90th Indian Science Congress*** held at Bangalore, during 3rd to 7th January, 2003
Year 2000	Qualified ***CSIR-NET JRF*** (National level examination for eligibility of Lectureship and Scholarship)
Year 2000	Qualified ***GATE*** (Graduate Aptitude Test of Engineering) with 93.96 percentile

Abstract/Publications

- **Jha A B,** Mishra S and Dubey R S. Effect of arsenic toxicity on antioxidant defense system in growing rice plants (Manuscript in preparation).
- Sharma P, **Jha A B,** Verma S and Dubey R S (2006) Induction of ascorbate and guaiacol specific peroxidases in metal and water deficit induced oxidative stress in rice seedlings. Physiology and Molecular Biology of Plants **12**: 1-10.
- **Jha A B** and Dubey R S (2005) Effect of arsenic on behaviour of enzymes of sugar metabolism in germinating rice seeds. Acta Physiologiae Plantarum **27**: 341-347.
- **Jha A B** and Dubey R S (2004) Carbohydrate Metabolism in Growing Rice Seedlings under Arsenic Toxicity. Journal of Plant Physiology **161**: 867-872.
- **Jha A B** and Dubey R S (2004) Arsenic exposure alters activity behaviour of key nitrogen assimilatory enzymes in growing rice plants. Plant Growth Regulation **43**: 159-168.
- **Jha A B** and Dubey R S (2004) Effect of arsenic toxicity on nitrogen assimilatory enzymes in germinating rice seeds. Indian Journal of Plant Physiology **9**: 438-441.
- Published an abstract on topic *"Effect of arsenic on enzymes of antioxidative defense system in growing rice plants"* in 92nd Indian Science Congress, Ahmedabad, 2005.
- Published an abstract on topic *"Effect of arsenic toxicity on behaviour of nitrogen assimilatory enzymes in growing rice plants"* in 91st Indian Science Congress, Chandigarh, 2004.
- Published an abstract on topic *"Arsenic toxicity induced alterations in sucrose and starch metabolism in growing rice plants"* in 90th Indian Science Congress, Bangalore, 2003.

Post Doctoral Research Summary

Project Title: "Enzyme purification, characterization and expression of enzymes of antioxidative defense system under arsenic toxicity"

Isoenzyme profiles and Immunoblot studies: When isoenzyme profiles of antioxidative defense system were examined by native PAGE followed by activity staining, As-led treatment led to increased intensity as well as appearance of new isozymes.
Immunoblot analysis of ascorbate peroxidase (APX) extracted from rice seedlings were conducted with antibodies raised in mice against cytosolic Euglena APX. Results of Immunoblot analysis of APX were quite similar to those obtained with the activities of corresponding isozyme.

Purification and Characterization of APX isoforms: Two isoforms of APX namely APX 1 and APX 2 were purified from rice seedlings using conventional biochemical purification

techniques of 33-66 % acetone fractionation, 40-90 % ammonium sulfate precipitation, dialysis and DEAE Sephacel column chromatography.

Two isoforms differ from each other in molecular and catalytic properties. APX 1 was purified to 41 fold with 4.4 % yield whereas APX 2 was purified to 232 fold with 17.4 % yield. Michaelis constant values of APX 2 and APX 2 for ascorbate were 382 μM and 195 μM respectively and for H_2O_2 were 30 μM and 71μM respectively. The activity of both isoforms declined by more than 65 percent by using compounds NADPH, NADH, guaiacol and glutathione as electron donors. Two isoforms showed a single band of protein after native and SDS-PAGE.

Project Title: "**Gene identification of mutant obtained by Tn5 mutagenesis of *Rhizobia"***

As a guest worker, I learnt the techniques of isolation of plasmid DNA from *E. Coli*; genomic DNA isolation from *Rhizobial* strains; purification of isolated genomic DNA; polymerase Chain Reaction (PCR), agarose gel electrophoresis; enzyme linked immuno sorbent assay (ELISA), 2-Dimensional polyacrylamide gel electrophoresis techniques.

PhD Research Summary

Topic: "Effect of arsenic toxicity on nitrogen assimilation, sugar metabolism and antioxidant system in rice plants"

Arsenic is a potential contaminant of groundwater in many parts of the world especially in Asian countries where higher soil arsenic level is attributed to excessive use of contaminated water for irrigating rice crop. The uptake of arsenic into crop plants is of greater concern, since this provides an entry point for the toxic element into the food chain. Therefore rice being a semiaquatic plant, suffers from increasing threat of arsenic toxicity. Though descriptions of visible plant injuries have been reported in certain plants, the detailed impacts of arsenic toxicity with special reference to the uptake and its influence on key metabolic processes in growing rice plants need to be examined in order to get an insight of the mechanism of phytotoxicity of arsenic in rice plants. With this objective my Ph.D. work was undertaken with the objectives to examine the effect of increasing levels of arsenic in the growth medium on its uptake, nitrogen assimilation, sugar metabolism and enzymes of antioxidant defense system in germinating seeds as well as in seedlings using two commonly grown rice *cvs*. Malviya-36 and Pant-12. For present studies, As_2O_3 solutions of 25 μM (3.7 ppm) and 50 μM (7.4 ppm) concentrations were served as treatment solutions.

Results:

Arsenic decreases the vigour of growing seedlings. Rice plants absorbed arsenic against the concentration gradient and the translocation of arsenic from roots to other organs is low. Arsenic inhibits the activities of key N assimilatory enzymes nitrate reductase, nitrite reductase, and glutamine synthetase and elevates the activities of glutamate dehydrogenases and aminotransferases (alanine and aspartate). Addition of 1 M proline, compatible cytoplasmic solute caused significant restoration in As-led loss of NR and GS activities. When experiments were performed to determine the Michaelis constant (Km) of NR, GS and GDH extracted from control grown and As-stressed seedlings, a significant increase in Km values of NR and GS and a decrease in Km of GDH were observed due to arsenic toxicity.

Arsenic level of 25 μM (3.7 ppm) and 50 μM in the growth medium caused an increase in the content of starch as well as reducing, non-reducing and total soluble sugars. Among starch degrading enzymes the activities of α-amylase and β-amylase were inhibited whereas elevation in the activity of starch phosphorylase was observed. The activity sucrose phosphate synthase declined whereas the activities of acid invertase, sucrose synthase were found to be elevated.

Arsenic treatment led to concomitant increase in the level of reactive oxygen species such as superoxide anion ($O_2^{\cdot-}$), thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2). Arsenic elevates the levels of the non-enzymic antioxidants ascorbate and

glutathione as well as enzymic antioxidants superoxide dismutase, catalase, guaiacol and ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase.

Conclusion:
In conclusion, inhibition in the activities of key N assimilatory enzymes might lead to an overall inhibition in the process of N assimilation whereas impairment in carbohydrate metabolism would influence photosynthate partitioning. These two events would ultimately contribute to impaired growth of rice seedlings in arsenic-polluted soils. Enzymes peroxidases and glutathione reductase appear to be important components of antioxidative defense mechanism in rice plants in combating arsenic-induced oxidative injury.

Importance:
Such studies will lead to developing strategies for improving stress tolerance and more specially heavy metal tolerance in plants. Further, the studies will lead to the development of specific, rapid and simple biochemical diagnostic field tests of plants in areas subjected to arsenic toxicity or other stresses.

References

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Personal Details

Date of Birth	07-04-1975
Sex	Male
Nationality	Indian
Marital Status	Unmarried