

Genomics & Molecular Biology group

1. Genomics of Arsenic Stress in Arsenic Accumulating Contrasting Rice Genotypes

One of the contributions from our research work is to understand molecular basis of differential As (Arsenic) accumulation in contrasting rice genotypes. Field trials conducted by our team, jointly with the Rice Research Station, Chinsurah (Hoogly, WB) for screening rice germplasm with respect to grain As accumulation showed major differences among accessions. Genome-wide expression study was performed in roots of low As accumulating rice genotypes (LARG; CN1646-5, NAYANMONI and CN1646-2) and high As accumulating rice genotypes (HARG; BRG-12, BRG-15 and BRG-20). Rice genotypes used in the study differ in tolerance and As accumulation in roots. Their abilities to respond to As stress showed up- or down-regulation of genes involved in various pathways and biological processes. This study suggests that the genetic mechanism regulating the differential As accumulation in different genotypes may not be dependent on gene expression at transcriptional level. However, many genes identified in this study, can be analyzed for marker-trait associations related to As accumulation in diverse genotypes around the world.

In an another study Cytochrome P450 *Os08g01480* gene and its promoter were cloned from *Oryza sativa indica sp.* (IR-64) and expressed in *Arabidopsis thaliana*. Transgenic lines of *Arabidopsis thaliana* showed early flowering and improved plant growth in comparison to NT. Transgenic plants were analysed for tolerance towards heavy metals as well as other abiotic stresses, which suggests *Os08g01480* has important role in providing tolerance to heavy metal stresses and other abiotic stress. Pathway studio analysis of co-regulated genes to identify common molecular connections between the proteins/transcription factors affected by *Os08g01480* which suggested that *Os08g01480* helps plant to combat arsenic stress including other environmental stress via auxin metabolism which is already established for its role in plant development and defense. Homozygous transgenic lines of *Arabidopsis* containing *Os08g01480* gene promoter shows its activity during heavy metal and abiotic stress. Analysis of Cytochrome P450 *Os08g01480* promoter study reflects the same response as of the transgenic lines of Cytochrome P450 *Os08g01480* in *Arabidopsis*. To sum up, all these studies indicate that the expression of cytochrome P450 *Os08g01480* is differentially modulated during different environmental stress as well as in different developmental stages.

2. Identification and Functional characterization of Class 1 Metallothioneins genes of Rice

Metallothioneins (MTs) are members of a family of cysteine rich low molecular weight polypeptides which play an important role in heavy metal detoxification and homeostasis of intracellular metal ions in plant. Though MT genes from some selected plants have been characterized with respect to their protein sequences, kinetic properties and tissue-specific localization, no detailed study has been carried out in rice. Here, we present genome-wide identification, structural and expression analyses of rice MT gene family. Our analysis suggests presence of eleven Class I MT genes in rice genome (Release 7 of the MSU Rice Genome Annotation Project) which are differentially expressed during growth and development, in various tissues and during biotic and abiotic stresses. Our analyses suggest that Class I MT proteins in rice differ in tissue localization as well as in heavy metal coordination chemistry. We also suggest that some MTs have a predominant role in detoxification of As (V) in arsenic tolerant rice cultivars. Our analysis suggests that apart from transcriptional regulation, post-transcriptional alternative splicing in some members of

this family takes place during growth and development, in various tissues and during biotic and abiotic stresses. Further characterization of heavy metal specific MTs are in the process.

3. Heterologous expression of *Ceratophyllum demersum* phytochelatin synthase, *CdPCS1*, in rice leads to lower arsenic accumulation in grain

Recent studies have identified rice (*Oryza sativa*) as a major dietary source of inorganic arsenic (As) and poses a significant human health risk. The predominant model for plant detoxification of heavy metals is complexation of heavy metals with phytochelatins (PCs), synthesized non-translationally by PC synthase (PCS). As a detoxification mechanism, heavy metals may be compartmentalized in vacuoles. In this study, in order to restrict As in the rice roots as a detoxification mechanism, a transgenic approach has been followed through expression of phytochelatin synthase, *CdPCS1*, from *Ceratophyllum demersum*, an aquatic As-accumulator plant. *CdPCS1* expressing rice transgenic lines showed marked increase in PCS activity and enhanced synthesis of PCs in comparison to non-transgenic plant. This enhanced metal accumulation potential of transgenic lines was positively correlated to the content of PCs, which also increased several-fold higher in transgenic lines. Transgenic lines showed enhanced accumulation of As in root and shoot. However, all the transgenic lines accumulated significantly lower As in grain and husk in comparison to non-transgenic plant. The higher level of PCs in transgenic plants relative to non-transgenic presumably allowed sequestering and detoxification of higher amounts of As in roots and shoots, thereby restricting its accumulation in grain.

4. Heavy metals induce oxidative stress and genome-wide modulation in transcriptome of rice root

Industrial growth, ecological disturbances and agricultural practices have contaminated the soil and water with many harmful compounds, including heavy metals. These heavy metals affect growth and development of plants as well as cause severe human health hazards through food chain contamination. In past, studies have been made to identify biochemical and molecular networks associated with heavy metal toxicity and uptake in plants. Studies suggested that most of the physiological and molecular processes affected by different heavy metals are similar to those affected by other abiotic stresses. To identify common and unique responses by different metals, we have studied biochemical and genome-wide modulation in transcriptome of rice (IR-64 cultivar) root after exposure to cadmium (Cd), arsenate [As(V)], lead (Pb) and chromium [Cr(VI)] in hydroponic condition. We observed that root tissue shows variable responses for antioxidant enzyme system for different heavy metals. Genome-wide expression analysis suggests variable number of genes differentially expressed in root in response to As(V), Cd, Pb and Cr(VI) stresses. In addition to unique genes, each heavy metal modulated expression of a large number of common genes. Study also identified *cis*-acting regions of the promoters which can be determinants for the modulated expression of the genes in response to different heavy metals. Our study advances understanding related to various processes and networks which might be responsible for heavy metal stresses, accumulation and detoxification.

5. Genome-Wide Transcriptome Analysis Reveals that Chromium Stress Signalling Controls the Expression of Genes in glutathione metabolism in Rice

Widespread use of chromium (Cr) contaminated fields due to careless and inappropriate management practices of effluent discharge, mostly from industries related to metallurgy, electroplating, production of paints and pigments, tanning, and wood preservation elevates its concentration in surface soil and eventually food chain becomes contaminated due to

accumulation in edible plant parts and causes several health hazards in humans as well as animals. In contrast to other heavy metals like cadmium, lead, mercury and arsenic, Cr has received little attention from plant scientists. Since Cr (VI) causes toxic effects directly or indirectly so deciphering the detoxification mechanism is often desirable to counteract such issues. Transcriptomics analysis revealed that the expression of several genes were modulated in roots by Cr (VI). Most of the genes differentially expressed were related to glutathione metabolism, transport, and signal-transduction pathways. However, somewhat unexpectedly, up-regulation of phytochelatin synthase was not detected in our transcriptome analysis suggesting that PCs are not involved in Cr (VI) detoxification. This might be due to their nonresponsive behavior to Cr (VI) stress, which is in agreement with previous report. On the contrary, in our study it is clear that glutathione plays an important role for detoxification of Cr-stress. Simultaneous analysis of microarray and metabolite content also suggested that sucrose degradation pathway was modulated in Cr stress response involving three main fermentation pathways operating as a rescue mechanism when respiration is arrested. In the subsequent study we identified two root specific Tau class GSTs (OsGSTU30 and OsGSTU41) which specifically up-regulated when exposed to Cr (VI). Overexpressing these two OsGSTs in *S. pombe* resulted in an enhanced resistance to Cr (VI) and OsGSTs-expressing yeast accumulated larger amounts of Cr (VI) than the vector control. Similarly, the transgenic yeasts also showed higher GST enzyme activities. Inhibition of glutathione biosynthesis by exogenous application of buthionine sulfoximine (BSO) abolishes the protective effect of OsGSTs against Cr (VI) stress which suggests a possibility that specific Tau class of GSTs are conserved in rice for binding with Cr (VI) and its homeostasis and detoxification.

6. Functional characterization of arsenate responsive glutaredoxin genes of Rice

We characterized two glutaredoxin (Grx) which shows highly up-regulation in previous microarray study results and probably involved in arsenic resistance in rice. We have cloned these genes in pET 28 b vector for expression of proteins, the expressed protein were confirmed by western blotting, and glutaredoxin specific HED assay. Further we transformed these construct (pEt28b-Os02g40500 and pEt28b-Os01g27140) in mutant *E. coli* strain and found that transformed AW 3110 (ars-1 Del) - ars operon deleted) showed about 2 mM of arsenate tolerance as compared to empty vector. And these glutaredoxins are also act like arsenite extrusion protein after transformation in JW 3469 (arsB758 Del) – arsenite extrusion protein).

For further characterization we transformed these two genes in *Arabidopsis* and rice.

7. Characterization of Novel arsenic methyltransferase from *Westerdykella aurantiaca* isolated from arsenic contaminated agricultural soil

Fungal strain, *Westerdykella aurantiaca* isolated from arsenic contaminated agricultural soils from West Bengal, India. This strain showed tolerance and methylation of arsenic when grown in mycological broth enriched with arsenic. Novel arsenic methyltransferase gene identified and cloned in yeast and ars mutants *E. coli* strains for its functional characterization. We found that transformed *E. coli* mutant strain (AW3110- ars operon is deleted) tolerate up to 2 mM of arsenate as compared to empty vector. For expression and purification of ASMT protein, ASMT gene was cloned with His₆ tag in *E. coli* strain BL21(DE3) and purified with His Gravitrap column. For further characterization we transformed this gene in *Arabidopsis* and rice.

8. Global Comparative molecular analysis to identify gene network regulating somatic embryo development in Japonica and Indica Rice varieties.

To initiate the work, an efficient protocol for somatic embryogenesis and regeneration in japonica rice has been established. Six different Checkpoints for differential expression analysis have been identified and each sample was harvested accordingly. RNA isolation from harvested samples has been carried out and RNA quality was checked using bio analyzer. After RNA integrity confirmation, samples have been sent for transcriptome sequencing. Until now sequencing has been done and data analysis is in progress.

Students

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Alumni

1. Manju Shri
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Recent Publications

2015

1. S Kumar, RS Dubey, RD Tripathi, **D Chakrabarty**, PK Trivedi (2015) Omics and biotechnology of arsenic stress and detoxification in plants: current updates and prospective. **Environmental International** 74, 221-230 (IF 5.6)

2014

1. Sonali Dubey, Manju Shria, Prashant Misra, Deepika Lakhwani, Sumit Kumar Bag, Mehar H Asif, Prabodh Kumar Trivedi, Rudro Deo Tripathi, **Debasis Chakrabarty** (2014) Heavy metals induce oxidative stress and genome-wide modulation in transcriptome of rice root. **Func Int Genomics**. 14:401–417 (IF 3.2)

2. Manju Shri1, Richa Dave, Sanjay Diwedi, Devesh Shukla, Ravi Kesari, Rudra Deo Tripathi, Prabodh Kumar Trivedi & **Debasis Chakrabarty (2014)** Heterologous expression of *Ceratophyllum demersum* phytochelatin synthase, *CdPCS1*, in rice leads to lower arsenic accumulation in grain. **Scientific Reports, 4 : 5784 DOI: 10.1038/srep05784 (Nature Publishing Group IF 5.01)**
3. Ankita Tripathi, Yuvraj Indoliya, Madhu Tiwari, Poonam Tiwari, Dipali Srivastava, Pankaj Kumar Verma, Shikha Verma, Neelam Gautam, **Debasis Chakrabarty (2014)** Transformed yeast (*Schizosaccharomyces pombe*) overexpressing rice Tau class glutathione S-transferase (OsGSTU30 and OsGSTU41) show enhanced resistance to hexavalent chromium. **Metallomics 6: 1549--1557 (IF 4.099)**
4. Amit Kumar, Rana Pratap Singh, Pradyumna Kumar Singh, Surabhi Awasthi, **Debasis Chakrabarty**; Prabodh Kumar Trivedi and Dr Rudra Deo Tripathi (2014) Selenium ameliorates arsenic induced oxidative stress through modulation of antioxidant enzymes and thiols in rice (*Oryza sativa* L.). **Ecotoxicology** (In press) (**IF 2.7**)

2013:

1. Smita Kumar, Mehar Hasan Asif, **Debasis Chakrabarty**, Rudra Deo Tripathi, Rama Shanker Dubey, Prabodh Kumar Trivedi (2013) Differential expression of rice lambda class GST gene family members during plant growth, development, and in response to stress conditions. **Plant Molecular Biology Reporter** 31:569–580 (**IF 5.3**)
2. Preeti Tripathi, Rudra Deo Tripathi, Rana Pratap Singh, Sanjay Dwivedi, **Debasis Chakrabarty**, Prabodh K Trivedi, Bijan Adhikari (2013) Arsenite tolerance in rice (*Oryza sativa* L.) involves coordinated role of metabolic pathways of thiols and amino acids. **Environmental Science and Pollution Research**. 20:884-896. (**IF 2.6**)
3. Richa Dave, Pradyumna Kumar Singh, Preeti Tripathi, Manju Shri, Garima Dixit, Sanjay Dwivedi, **Debasis Chakrabarty**, Prabodh Kumar Trivedi, Yogesh Kumar Sharma, Om Prakash Dhankher, Francisco Javier Corpas, Juan B Barroso, Rudra Deo Tripath (2013) Arsenite Tolerance is Related to Proportional Thiolic Metabolite Synthesis in Rice (*Oryza sativa* L.). **Archives of environmental contamination and toxicology**. 64:235-242 (**IF 2.0**)
4. Smita Kumar, Mehar Hasan Asif, **Debasis Chakrabarty**, Rudra Deo Tripathi, Rama Shanker Dubey, Prabodh Kumar Trivedi (2013) Expression of a rice Lambda class of

glutathione S-transferase, OsGSTL2 in Arabidopsis provides tolerance to heavy metal and other abiotic stresses. **Journal of hazardous materials.** 248:228-237. (IF 3.9)

5. Preeti Tripathi, Rudra Deo Tripathi, Rana Pratap Singh, Sanjay Dwivedi, Dheeraj Goutam, Manju Shri, Prabodh K Trivedi, **Debasis Chakrabarty (2013)** Silicon mediates arsenic tolerance in rice (*Oryza sativa* L.) through lowering of arsenic uptake and improved antioxidant defence system. **Ecological Engineering.** 52:96-103 (IF 2.9)
6. Dave R, Tripathi RD, Dwivedi S, Tripathi P, Dixit G, Sharma YK, Trivedi PK, Corpas FJ, Barroso JB, **Chakrabarty D (2013)** Arsenate and arsenite exposure modulate antioxidants and amino acids in contrasting arsenic accumulating rice (*Oryza sativa* L.) genotypes. **Journal of Hazardous Materials.** 262 (2013) 1123–113. (IF 3.9)

Annexure-III

1.5. Highlight of the significance/impact of your work on industry/societal/environmental/nation as a whole (not more than 100 words).

Arsenic (As) contamination of the environment has emerged as a serious problem. Consequently, there is an urge to understand plants' responses to As. Our study offers a hope for understanding the molecular basis of arsenic toxicity and accumulation in plant parts. Although measurable success, in terms of application in the field, has so far not been achieved, transgenic research has yielded promising results, which shed light on the approaches to be taken up in future endeavour. This will have tremendous societal impact related to public health consequences.