NAME OF THE SCHOLAR : MOHD ANWAR AHMAD
NAME OF THE SUPERVISOR: DR. MEETU GUPTA
DEPARTMENT/FACULTY : BIOTECHNOLOGY/NATURAL SCIENCES
JAMIA MILLIA ISLAMIA, NEW DELHI-25

TITLE OF THE THESIS: CELLULAR/MOLECULAR MECHANISMS INVOLVED FOR ARSENIC DETOXIFICATION AND TOLERANCE IN RICE AND INDIAN MUSTARD VARIETIES

ABSTRACT

Mobilization and accumulation of arsenic in different plant parts is a serious threat to human populations world-wide. Several processes are involved in accumulation and homeostasis of As to regulate its uptake, transport and distribution in different cells and tissues. Various reports and analyses indicates that thiol containing peptides, glutathione, antioxidant defense system and metal tolerant genes involves in the detoxification of As species. However, not many studies have been carried out to see the comparative evaluation of different As species tolerant and sensitive crops and other plant varieties to select safer crops/plants for human consumption and also for phytoremediation purposes. The major goal of this study was to evaluate the differential response of As species in two varieties of *Brassica juncea* (Indian Mustard) and *Oryza sativa* (Rice) as *Brassica* is known as good metal accumulator and rice is the main staple food in As contaminated areas. With this goal, experiments were designed to see the responses of different varieties of both plants at physiological, biochemical and molecular level and the results are summarized under following headings:

1) Study of apparent toxicity symptoms under arsenic stress including seed germination, root-shoot length with some basic physiological parameters (chlorophyll and protein.)

In this study, germination of both mustard and rice seed varieties were found to be affected considerably with As species and their concentrations after 7 days. Percentage seed germination and root-shoot length (cm) inhibited as the concentration increases in both the varieties of mustard (PB & PJn) and rice (PBI & IR-64). However, inhibition was more in PJn and IR-64. variety of mustard and rice, respectively. As(III) species was found to be more toxic as compared to As(V) in terms of toxicity for both plants.Similar pattern was observed for chlorophyll and protein content as maximum reduction was observed at higher concentration (300 μM) and duration (96h) of As species in both varieties of mustard and rice plant. More inhibition of both content was found in PJn and and IR-64 variety. Reduction in chlorophyll and protein content was more under As(III) stress at all tested concentration.

2) Accumulation pattern of Arsenic (As(III) & As(V)) in rice and Indian mustard varieties at different time intervals.

A differential rate of As accumulation was observed for both As species. Irrespective of the As species, the accumulated concentrations in both root and leaves increased with increasing As concentration and followed the trend (i) As(III) > As (V) (ii); root > leaves in the varieties of both plants. PB1 variety of rice and PB of mustard showed more accumulation under stress of both As species as compared to IR-64. Translocation factor shows the efficiency of plant to

transport metal from root to shoot or leaves. Our results showed more TF in case of As(III) as compared to As(V) in all varieties except in case of rice var. IR-64 for 24h duration.

Overall, *Brassica* varieties accumulated more As as compared to rice varieties.

3) Analysis of biochemical changes (SOD, CAT, APX, Cysteine, Proline and Malonaldehyde content) under arsenic stress

Results of the study presents evidence that As species triggers excessive generation of O_2 and H_2O_2 and induces lipid peroxidation. Though, both As species known to stimulate generation of ROS, plants respond differently to As(III) and As(V). Increase in the activities of antioxidative enzymes (SOD, CAT and APX) and stress related parameters (cysteine, proline and MDA) in the leaves were observed in varieties of both plants, being more in PB and PB1 variety of mustard and rice, respectively under As(III) stress. All varieties showed maximum increase of all parameters at 150 μM As(III)/(V) as compared to other tested concentrations.

4) Induction and suppression of proteins (SDS-PAGE) in As sequestration

Comparing the profiles of control and As treated leaves of all varieties significant changes were observed in the pattern of protein ranging from 19 – 118 KDa. However, in both mustard and rice varieties, under As(III) and As(V) stress, 50 KDa protein was more affected as compare to their control after 24 h duration, but its intensity increased at 300 μM at 96 h duration. More changes were observed in case of As(III) treated leaves as compared to As(V). Furthermore, intensity of bands were found to be more affected in PJn and IR-64 variety.

5) Transcript profiles of genes related with metal binding peptides (PCS, MT-2, GR and GS) under As stress.

The molecular responses by semi-quantitative RT-PCR in leaves of hydroponically grown varieties of mustard and rice plants under As species stress, were assessed by transcript accumulation of genes (*PCS*, *MT*-2, *GR* & *GS*) responsible in metal tolerance. Both species of As induced higher gene expression of all the studied genes. In case of both rice and mustard plant, As(III) treatment , in general, induced greater transcript accumulation as compared to As(V) and PB1 and PB variety showed more up-regulation of transcripts, respectively, at higher concentrations and duration of As species as compared to IR-64 and PJn. It is important to note that up-regulation or down regulation of these genes were more affected at 150μM As(III) and at 300uM As(V) after 96 h duration. These findings are consistent as other stress related parameters also showed more enzyme activity at 150uM As(III) as compared to As(V). Overall level of gene transcripts, antioxidative enzymes and other stress related parameters showed a combinatorial type of tolerance mechanism in tolerant varieties (PB & PB1) to provide protection against As(III) and As(V) stress in all the tested varieties.

In conclusion, in the present study, opposite patterns of response to As species in *Brassica* and *Oryza* varieties were obtained as judged by enhanced activities of antioxidative enzymes, stress related parameters and metal tolerant genes along with some basic toxicity parameters. Better As stress tolerance in Pusa Bold (mustard) and Pusa Basmati (rice) variety was associated with its ability to maintain higher induced activities of SOD, CAT, APX, Cysteine and Proline along with more transcript accumulation of metal tolerant genes.