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ABSTRACT

Title: Combinatorial Effect of Arsenic and Selenium Interaction on Growth and Development of Rice

Rice consumption by the world population is a major dietary source of inorganic arsenic (As), a non-essential and carcinogenic metalloid that causes food safety problem. Depending on environmental factors, As accumulation in food crops may pose major health threats to the millions of people relying on rice as their subsequent diet (Gousul Azam et al. 2016). Any direct or indirect damage in plants alters the nutrient uptake and transport. Arsenic is known to affect the plant adversely and alters the nutrient content, which are essential for human health. While selenium (Se) is considered as an essential element for humans and animals shares similar chemical properties with As, therefore, biological interactions between As and Se may affect the functioning of plants antagonistically or synergistically. Presence of Se supports the expression of variety of selenoproteins, which have important antioxidant role and detoxification function. Hence, Se mediated detoxification pathways have great importance in triggering As detoxification in plants. The present study may shed light for a better understanding of the As accumulation; role of toxicity indicators, modulators and sulfur-related gene transcripts, nutrient profiling, expression analysis of genes involved in the absorption and utilization of N, P, K, and molecular mechanisms of plant response during As-Se interaction.

In the present study, two rice var. PB1 and IR64 were selected to see the interactive effect of As and Se. The results are summarized under following headings:

<u>Study of apparent toxicity symptoms and accumulation of As and Se during As and Se</u> <u>interaction</u>

Findings revealed that Se played protective role in both IR64 and PB1 varieties, however, growth parameters, chlorophyll and protein content was better in IR64 as compared to PB1 during combined treatment of As and Se.

As accumulation was more in PB1 than IR64 under As and As+Se treatment, although, the reduction percentage for As accumulation in PB1 was less than IR64 in As+Se treatment, when compared with As alone treatment. Selenium accumulation was decreased in As+Se treated seedlings of both the varieties as compared to those grown only with Se.

<u>Alleviation of As stress in Oryza sativa var. PB1 and IR64 by Se through combining the</u> <u>role of toxicity indicators, modulators and sulfur-related gene transcript</u>

Increase in the activities of antioxidative enzymes (SOD, CAT, APX) and toxicity indicators (MDA & H_2O_2) were observed during As stress, which were further decreased during Se supplementation, due to less production of ROS.

Content of stress modulators (proline, cysteine) were increased during co-application of Se to As stressed varieties due to alleviating properties of Se.

Selenium supplementation also significantly affected the thiol content in both leaves and roots, and modulated the gene expression level of sulfur related gene transcripts (APS, MT-2, GR, GS, Sultr 4,1). Arsenic and Se affected the sulfur metabolism at assimilation, transportation, and enzymatic (GR, GST) activities.

Overall, IR64 performed better during As-Se interaction as compared to PB1.

Nutrient profiling and expression analysis of genes involved in the absorption and utilization of N, P, K

NPK content in both varieties were positively correlated with the differential gene regulation pattern of N, P and K, which plays role in metabolism and nutrient availability in both varieties.

Overall, both the varieties when compared, As susceptible variety IR64 performed better in terms of nutrient balance and growth under As+Se combination as compared to PB1, which signifies the protective role of Se during As stress.

miRNA plays role in the antagonistic effect of Se on As stress

Differential regulation pattern of at least 46 miRNAs in rice seedlings were obtained as compared to untreated control. Eighteen of these miRNAs showed differential regulation among different treatments and these miRNAs have important role in abiotic stress.

Differentially regulated miRNAs and their identified cis-acting element and targets revealed their role in stress tolerance.

In conclusion, this study highlights the important role of Se on As accumulation and metabolism in leaves and roots of two rice var. PB1 and IR64. Selenium supplementation decreased As accumulation in both varieties, however more accumulation was observed in PB1 variety. Better growth performance was observed through various morphological, physiological and biochemical parameters in both varieties during co-application of Se to As. Further, this study identified the amount of nutrients and their transportation during individual treatment of As, Se and in their combined treatments. Results revealed that Se mitigates the effect of As by improving nutrient content, and positively correlated with the differential gene regulation pattern of N, P and K, which plays role in metabolism and nutrient availability in both varieties. Various miRNAs and their role in metal interaction were identified through microarray analysis of miRNAs. On the basis of combinatorial studies, As toxicity was found to be ameliorated in the presence of Se in both rice varieties. PB1 variety showed higher tolerance to As while IR64 variety was able to overcome As toxicity in a better manner than PB1 on Se supplementation i.e. IR64 performed better with Se.