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Title of thesis: Biochemical and molecular analysis of silicon mediated changes during Arsenic induced stress in rice (Oryza sativa L.)

Abstract

Title: Biochemical and molecular analysis of silicon mediated changes during Arsenic induced stress in rice (Oryza sativa L.)

Rice is staple crop for half of the world's population. The average consumption of rice is about 200-600 g/day/person. Inorganic forms of arsenic (As) are class one carcinogenic metalloid, toxic to all forms of life. Being a staple crop, production rice must be sustainable to fulfil the global demand and must have low As content. As contamination in paddy soil not only affects the rice growth, development and yield but also affects the human health, since rice is a staple crop and serve as a major dietary source of As in human after drinking water. Macro and micro nutrients are essential for plants growth and development. While, As is known to affects nutrient transport and accumulation in plants (Wang et al. 2010). Silicon (Si) is quasi essential mineral nutrient for plant, however, their presence in the plant growth medium results enhancement in growth as well as the yield. Si is considered as beneficial for plant particularly under biotic and abiotic stress (Epstein 2009). Si had shown to increase the nutrient availability for the plant. Biotic and abiotic stress leading to increase in oxidative stress by uncontrol generation of Reactive Oxygen Species (ROS). Si fertilization effectively releases the oxidative stress by maintaining the over production of ROS by enhancing antioxidants (Etesami and Jeong 2018). Silicic acid and $As^{(III)}$ have similar size with same pKa; due similar size both have taken up by Silicon transporter (*OsLsi*) in rice. Both Si and $As^{(III)}$ markedly regulated the expression of *OsLsi,* however, Si restrict the passage of As in the plant when supplied both in combination. The present study may shed the light for better understanding of As accumulation and toxicity such as nutrient accumulation, expression of nutrient (N, P and K) transporters genes, toxic indicators, antioxidant activities, and genotoxic effects under Si supplementation.

In the present study, rice var. IR64 was selected to see the effect of As and Si at 7d and 15d duration. The results are summarized under following headings:

Study of evident toxicity symptoms induced by $As^{(III)}$ including seed germination, root-shoot length with some basic physiological parameters (chlorophyll and protein) and its mitigation by Si.

As toxicity showed adverse effects on plant growth as plant showed decreased shoot and root length. the result also showed decrease in chlorophyll and protein content in both shoots and roots at both duration (7 and 15 days) being more at 15 days. The result revealed that Si enhanced seed gemination rate and growth of plants also enhanced chlorophyll and protein content hence showing the protective role of Si during As(III) stress.

Accumulation pattern of As and Si and gene expression of Si transporter

The protective role of Si during $As^{(III)}$ stress is apparent by decreasing As content in $As^{(III)}$+Si treated plants. As accumulation As was more in roots than shoots at both durations in $As^{(III)}$+Si, however,

the reduction percentage in roots for As was less than shoots at both durations. The expression of Si transporter was higher in $As^{(III)}$+Si treated plants than $As^{(III)}$ only, being more in the shoots than roots. The presented findings could be useful for engineering to alter the ability of *Oryza sativa* varieties regarding As accumulation pattern by using Si containing fertilizer, depending on rice genotypes and irrigation practices.

Alleviation of As stress in rice var. IR64 by Si through combining the role of toxicity indicators and modulators

In the present study result showed that As stress triggers the excessive generation of superoxide radicals, peroxides and induces lipid peroxidation. Silicon (Si) reduced the generation of ROS (H_2O_2 and $O_2^{\cdot -}$) at both duration more in roots than shoots, comparing to control. Increase in the activities of antioxidative enzymes and toxicity indicators (MDA & H_2O_2) were observed during As stress, which were further enhanced during Si supplementation, subsequently reduced H_2O_2 content. Content of proline and cysteine (stress modulators) were decreased during co-application of Si with $As^{(III)}$ at both durations in both shoots and roots. Overall, enzymatic activity was more in $As^{(III)}$ which was further enhanced by application of $As^{(III)}$+Si. This indicates that Si is able to mitigate As stress when supplemented with Si.

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

Application of Si alleviates the deteriorating effect of $As^{(III)}$ by improving nutrient content. $As^{(III)}$+Si mediated upregulation of genes of N, P and K, over $As^{(III)}$ was positively corelated with their respective nutrient contents. The expression pattern of genes clearly suggested that these genes might play diverse roles in the oxidative stress, growth and developmental processes, and nutrient absorption processes under $As^{(III)}$ stress. Overall, at both durations when compared $As^{(III)}$ and $As^{(III)}$+Si, plant treated with $As^{(III)}$+Si perform better in terms of nutrient balance and growth as compared to $As^{(III)}$, which suggests the protective role of Si during As stress.

Si lessened genotoxic alteration caused by $As^{(III)}$

In the current study, an effort has been made to study the RAPD profile by a combination of As and Si in rice seedlings. Analysis of the RAPD profile showed that Si playing major role in lessening of genotoxicity caused by $As^{(III)}$ in terms of GTS (genomic templet stability). Si application lowered percentage polymorphism at both durations (7d and 15d) in both tissue (shoots and roots) which was increased due to $As^{(III)}$. The change in the GTS(%) and polymorphism in $As^{(III)}$+Si treatment are positively corelated with other growth parameters like germination percentage, plant height, chlorophyll, protein content, lower MDA and decreased activity of LOX.

In conclusion, this study highlights the important role of Si on As accumulation and metabolism in shoots and roots at two time periods (7day and 15day). Silicon supplementation decreased As accumulation in both tissue at both durations, maximum decrease was observed in shoots than roots comparing to $As^{(III)}$. Si supplementation along with $As^{(III)}$ results better growth in terms of morphological, physiological and biochemical parameters at both durations. Further, this study identified the amount of nutrients and their transportation during individual treatment of Si and As and in their combined treatments. Results revealed that Si 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. Si along with As plays important role to diminish genotoxicity induced by As. Overall, results showed that the application of Si along with As reduced the overall toxicity by decreasing As content and improved plant growth and nutrient contents by regulating the defensive enzyme and nutrient transporters.