



Journal Homepage: -www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI:10.21474/IJAR01/8239
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/8239>



RESEARCH ARTICLE

EFFECTS OF ARSENIC AND THEIR MITIGATION IN PLANTS.

Kanta bokaria.

Food Technology Department, Gurunananak Institute Of Technology, 157/F Nilgunj Road, Sodepur West Bengal, Kolkata-700114, India, 9433613947.

Manuscript Info

Manuscript History

Received: 18 October 2018

Final Accepted: 20 November 2018

Published: December 2018

Key words:-

Arsenic, Environment, hydrological cycle, metals, chromosomal damage.

Abstract

Arsenic is a metalloid - a natural element that is not actually a metal but which has both the properties of a metal and a non metal. It is a natural component of the Earth's crust, generally found in trace quantities in all rock, soil, water and air. However, concentrations may be higher in certain areas due to either natural conditions or human activities. Soil contaminants—organic like petroleum, hydro-carbon, fertilizer, pesticide, copper, nickel cobalt zinc, Inorganic like arsenic cadmium, mercury, lead, Arsenic (As) is posing a serious health concern in West Bengal in India. Long term Arsenic exposure leads to skin lesions and various types of cancers. Safe level of As in drinking water is $10 \mu\text{g l}^{-1}$, as recommended by World Health Organization in 1993, while the level of As in ground water has been reported up to $3200 \mu\text{g l}^{-1}$ in West Bengal. The total daily intake should not exceed 2 mg of inorganic arsenic per kilogram of body weight. Arsenic is non-essential element for plant and present in environment both in inorganic as well as organic forms. Arsenate (As^{V}) and arsenite (As^{III}) are predominant inorganic forms. As toxicity symptoms in plants range from inhibition of root growth, photosynthesis to death of plant. Arsenate shows structural analogy with phosphate so it is mainly transported through high affinity phosphate transporters.

Copy Right, IJAR, 2017.. All rights reserved.

Introduction:-

To reduce global crisis of food security by producing arsenic free Food and water, by making arsenic ineffective or stopping arsenic to move to plants. Arsenic is chemically similar to phosphorus an essential plant nutrient so can be substituted for phosphorus as a fertilizer.

Presence of As in grains hampers the nutritional value of rice in terms of trace nutrients and amino acids (Kumar et al., 2014a). Arsenate (As^{V}) and arsenite (As^{III}) are predominant inorganic forms. Arsenate shows structural analogy with phosphate so it is mainly transported through high affinity phosphate transporters (Tripathi et al., 2007). Salicylic acid (SA) is an important signaling molecule and plays a crucial role in resistance against biotic and abiotic stress in plants. In present study, ameliorative effect of SA against arsenate (As^{V}) toxicity has been investigated in rice (*Oryza sativa* L.). Arsenate stress hampered the plant growth in terms of root, shoots length, and biomass as well as it enhanced the level of H_2O_2 in paddy field, As^{III} is the predominant chemical species of As due to anaerobic growing conditions (Takahashi et al., 2004) As is a redox active metalloid and induces the generation of reactive oxygen species (ROS) leading to lipid peroxidation, disruption of cellular redox state, and associated

Corresponding Author:-Kanta bokaria.

Address:-Food Technology Department, Gurunananak Institute Of Technology, 157/F Nilgunj Road, Sodepur West Bengal, Kolkata-700114, India, 9433613947.

toxicity (Finnegan and Chen, 2012). This study is hypothesized to investigate positive impact of SA on As^V tolerance in rice. Arsenic was identified by the German alchemist Albertus Magnus around 1250 AD. Arsenic was used by the ancient Asians, Egyptians, Greeks, Romans and Chinese. In 1649 Schroeder published two methods of preparing the element. Arsenic is a highly poisonous metallic element. It has three allotropic forms, yellow, black, and grey. Its name comes from the word arsenic on which is the Greek name for the pigment yellow or pigment. Some of the Arsenic are useful also for wood and leather preservation, polishing and medicinal purposes. The following are some of the most common uses for arsenic in the world today (Benner, 2010). The arsenic is used as pigment in paints. Specially as a white extender pigment in white paints where whiteness was important. It is also used to enhance green pigments. The main use of metallic arsenic is for strengthening the alloys of copper and lead to use in batteries and other purposes. Arsenic is also used in numerous pesticides, herbicides and insecticides though this practice is becoming less common as most of these products are banned. It is used as a wood preservative because of its toxicity to insects, bacteria and fungi. Arsenic is used in the medical treatment of cancers such as acute promyelocytic leukemia. Arsenic-74 an isotope is being used as a way to locate tumors within the body. It produces clearer pictures than that of iodine.

Toxicity of arsenic:-

The toxicity and deleterious effects of arsenic on living being as well as green environments are well documented. Long term drinking water exposure causes skin, lung, bladder, cardiovascular diseases, and kidney cancer as well as pigmentation changes, skin thickening (hyperkeratosis), neurological disorders, muscular weakness, loss of appetite, and nausea (Zhao et al., 2012; Wang et al., 2007; Guha Mazumder, 2007; Kapaj et al., 2006; Kitchin and Wallace, 2008). Toxicity differs between various arsenic compounds, for example, monomethyl arsenic acid and inorganic arsenide have a higher toxicity level than arsenic choline. Acute toxicity is generally higher for inorganic arsenic compounds than for organic arsenic compounds (Sharma and Sohn, 2009). Oral intake of more than 100 mg is lethal. The lethal dose of arsenic trioxide is 10-180 mg, and for arsenide this is 70-210 mg. The mechanism of toxicity is binding and blocking sulphur enzymes. Arsenic pollution has been reported recently in the USA, China, Chile, Bangladesh, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, New Zealand, Japan and India. The WHO provisional guideline of 10 ppb (0.01 mg/L) has been adopted as the drinking water standard. However, many countries have retained the earlier WHO guideline of 50 ppb (0.05 mg/L) as their standard or as an interim target including Bangladesh and China. In 2001, US-EPA published a new 10 ppb (0.01 mg/L) standard for arsenic in drinking water, requiring public water supplies to reduce arsenic from 50 ppb (0.05 mg/L) to 10 ppb (USEPA, 2001), which are in effect from January 2006. Arsenic toxicity is known to interfere with sulfhydryl groups in cells of most plants. Hence, plants those are not tolerant to arsenic shows toxic symptoms such as a decrease in plant growth, plasmolysis, wilting and necrosis of leaf tips, and decrease in photosynthetic capacity

Methodology:-

Thirty one rice genotypes comprising of popular cultivars, land races adopted for up land and low land ecology, were grown in P-sufficient soil (available P- 60 mg/kg), experimental Farm, and P-deficient soil (available P< 3.50 mg/kg) of research station for Red and Lateritic zone in the kharif season To estimate soil phosphate, bunding was properly made surrounding the field to prevent any invasion of phosphate through other irrigation channels .Recommended fertilizer dose of NPK was applied for plant sample (mature shoot) with three replications for each line was done with Agilent 8453 spectrophotometer after tri-acid digestion .For the Arsenic physical chemical measurement Soil samples were collected from the three different points o f soil surface (0-20 cm) from the seed Research Farm at Chakdha This soil was contaminated previously with arsenic through urban sewage sludge. The samples were mixed, transferred to the laboratory, passed through 2 mm sieve and used for physical-chemical and microbial analysis. One gram of soil sample was placed in the nickel plate that already covered its bottom with NaOH. Heated the Nickel plate in order to fully melt NaOH and mixed with soil (alkaline digestion). After cooling, the nickel plate was immersed in HCL (0.5 N) and waited in order to digest slowly then the amount of arsenite was measured. To measure arsenic the spectrophotometer method was used along with a reagent called Leuco malachite green (LMG). one replicate beaker containing 15–20 seedlings at six concentrations of arsenate will be used to characterize the dose–response for varieties The data for each plant in a beaker will be averaged and the standard error was calculated. In this method arsenic reacts with Potassium iodate (KIO₃) in the acidic environment and iodine will be released. Released iodine oxidizes LMG to MG and changes the colour to the colour of malachite green. Detection range of arsenic concentration in this method is 0.09-0.9 micro g/ml. The MG dye shows maximum absorption at 617 nm. Analysis of Pup1-K42 and Pup1-K29 genes using PCR. DNA was extracted from approximately 40 mg of fresh leaf tissue,. Standard PCR was carried out using thermal cycler (Gene Amp PCR System 9700). The reaction volume (25 ml) contained diluted DNA sample 20ng with 100 ng each of forward and

reverse primer, along with 2.5 ml 10X buffer, 2.0 mM MgCl₂ solution, 1 ml 2.5mM dNTPs, 16.5 ml HPLC grade sterile water and 0.5 U Taq DNA polymerase enzyme (Chromus Biotech). Amplification was carried out with the reaction condition of 94°C for 5 minutes of initial denaturation followed by 35 cycles each of denaturation at 94°C temperature for 45 seconds, annealing at 58°C and polymerization and PCR products were size fractionated in 1 % Agarose gels and stained with ethidium bromide and documented.

Growth Conditions and Experimental Design:-

Seeds collected from BCKVV seed Research Farm Kalyani, West Bengal (India), were surface sterilized using 10% H₂O₂ for 30 s and washed with Milli Q water. Seeds were germinated on moist pre-sterilized blotting sheets in a tray, placed in seed germinator for 4 days at 25°C, relative humidity was 65%. After 7 days, 50 uniform size seedlings were selected and placed in 150 ml beakers, covered with black sheet, containing 100 ml of 100% Hewitt nutrient medium, prepared in Milli-Q water (pH 6.8–7.0) and grown for another 10 days under light intensity 210 $\mu\text{M cm}^{-2}\text{s}^{-1}$ (16/8 h; day/night). 10 days old plants were provided As^V (25 and 50 μM) using the salt Na₂HAsO₄ and SA (100 μM) in the nutrient medium and grown for 7 days. Plants treated by 25 and 50 μM As^V, 100 μM SA for 7 days abbreviated as As^V25, As^V50 and SA, respectively. Plants treated with As^V25, As^V50 supplemented with SA abbreviated as SA + As^V25 and SA + As^V50. For Pre-treatment of SA, plants were grown in 100 μM SA for 3 days and then transferred to Hewitt solution containing As^V25, As^V50 for 7 days. Plants grown in As^V deprived medium termed as SA Pre and plants grown only in Hewitt solution served as control.

Element Estimation:-

The elements (As and Fe) content was determined following Mallick et al. (2012). Briefly, plant tissues were washed three times with Milli Q water and plants separated in root and shoot and oven dried at 70°C. Dried plant tissues (root 300 and shoot 500 mg) were digested in HNO₃: HCl (3:1). Digested samples were filtered through Whatman filter paper 42 and volume was made to 10 ml by Milli-Q water. As and Fe were estimated by using AAS (GBC Avanta S, USA) fitted with a hydride generator (MDS 2000) using NaH₂BO₄+NaOH (3 M) and HCl (3 M). The values were presented in μg per gram dry weight ($\mu\text{g g}^{-1}\text{dw}$).

Endogenous Salicylic Acid Estimation:-

Presence of SA in shoot samples were analyzed by HPLC (Dionex Ultimate 3000) using UV detector at 210 nm by following the method of Pan et al. (2010). The mobile phase was programmed with linear gradient of A (0.1% of formic acid in methanol) and B (0.1% of formic acid in water) as 0–20 min; 30–100% A, 20–22 min; 100% A and then 22–25 min; 100–30% of A. Flow rate was maintained at 0.3 ml min⁻¹. Retention time for SA was recorded at 22.4 min.

Statistical Analysis and Analytical Quality Control:-

The whole experiment was set up in the randomized block design. The data were subjected to Duncan's Multiple Range Test (DMRT) for the analysis of significant difference between the treatments.
Results and Discussion

Phenotyping of P-uptake ability in field grown plants:-

P-uptake in rice plants was measured at maturity and expressed in mg per plant. P-uptake ability in deficient soil was measured when they grown in red and lateritic soil of West Bengal where available P was less than 3.5 mg/kg. P-uptake ability of the same set of plants was measured in P-sufficient soil when they were grown in alluvial soil where available P is 60 mg/kg. P-acquisition efficiency varied significantly among the genotypes both in P-deficient and sufficient soil. Genotypes with high P-uptake ability have significantly ($p < 0.01$) higher dry-mass-weight, 30.72 mg plant⁻¹, than that of average 21.66 mg plant⁻¹. Like P-sufficient soil, a significant correlation between P-uptake and dry-mass-weight ($r = 0.81$) of the rice genotypes was found in P-deficient soil. Genotypes like, Gitanjali, Gobindabhog, Jaladhi, Pusa Saugandh, Radhunipagol, Tulaipanji may be considered as donor parents where P-acquisition efficiency both in P-limiting and non-limiting condition was higher than the average. The rice plants accumulated significant amount of As in upon exposure to As^V in dose dependant manner. In all treatments more than 90% of As was confined in to the roots. SA co-application to As^V treated plants had no significant impact on As accumulation in root was observed. Accumulation of Arsenic (As) and Fe in the root and shoot of **Oryza sativa** after 7 days of treatment with different combinations of As^V and SA. Arsenate treatment significantly enhanced total Fe accumulation in comparison to control plants. The translocation of Fe to shoot was reduced drastically in As^V treated plants which was lower than control shoot. Co-application as pre-treatment of SA reduced the total Fe accumulation in comparison to As^V alone, however, its translocation to shoots increased

significantly in shoots comparable to control. The results of different tests undertaken were more interesting as we found measurable amounts of total arsenic in its two forms in all the rice varieties undertaken for study. For this experiment soil sample were collected from 15 farmers field of Chakdha, of NADIA district of West Bengal research farm, comparison of seed quality by germination test in the field and in the lab was done and agronomy characters are measured and compared to find the best arsenic resistant variety (TABLE 1.)

Table 1:-

S No	Name	Village	Dist	Dag No	Arsenic in the soil sample	Mineral & Salt	P H	Carb on %	Phosphorus	Potassium
1	Haran Chandra Das	Phetugachi	Nadia	Jodkhambba	.3171	0.16	8.14	High	Low	Low
2	Narayan C Das	Phetugachi	Nadia	Uparer Phali	.3178	0.13	8.18	Low	Medium	Low
3	Badsha Mondal	Sajerdhar	Nadia	Sajerdhar	.3166	0.11	8.07	Low	Low	Low
4	Badsha Mondal	Chasadhopapara	Nadia	Sajerdhar	.3174	0.14	8.14	Low	Low	Low
5	KRISHNA GOPAL DAS	MOHISH DANGA	Nadia	PURBODIPER PHALI	.3175	0.19	8.22	Low	Low	Low
6	KRISHNA GOPAL DAS	MOHISH DANGA	Nadia	PASCHIM DIPER MOMI	.3168	0.12	8.2	Low	Low	Low
7	MADHAV DAS	MOHISH DANGA	Nadia	DHIJAN DASER	.3173	0.1	8.24	Medium	Medium	Low
8	MADHAV DAS	MOHISH DANGA	Nadia	16 SATAK	.3165	0.13	8.31	Medium	Medium	Low
9	MADHAV DAS	MOHISH DANGA	Nadia	ACHEYDER JAMIN	.3179	0.18	8.15	Low	Medium	Low

The four most common varieties used in arsenic affected area of West Bengal showed significant amount of accumulated As upon exposure to As^V in dose dependant manner. In all treatments more than 90% of As was confined in to the roots and had no significant impact on As accumulation in root. However, the shoot As was reduced significantly, plants was observe less As in shoot upon exposure to 25 and 50 μ M As^V, respectively, salicylic acid serves as an important signaling molecule in plant system which has been shown to play role in against heavy metal toxicity. The co-application and pre-treatment of SA with As was used to investigate persistence of signaling aspects of SA. Arsenic is well known to adversely affect the plant growth and development upon its accumulation (Kumar et al., 2015). A significant amount of As was accumulated by the rice plant that hampered the plant growth severely. Application of SA, either co- or pre- treatment with As^V has significantly reduced the total accumulation of As (Root + shoot) with more reduction in the shoot. Though, the co-application of SA was more effective in reducing As accumulation than pre-treatment of SA. Thus, SA treatment has negatively impacted the root to shoot translocation of As. This might be due to SA-mediated down regulation of root to shoot as transporters. The harmful effects of arsenic and how to control arsenic uptake in Plants and animals, so that people are saved from being sick by different serious diseases caused due to presence of arsenic. Chronic arsenic poisoning is due to continuous exposure to arsenic compounds, like arsenides, sulfides, and sulfosalts in natural environment which leads to an accumulation of arsenic in the body and causes many harmful diseases to humans, plants and animals as high level of Arsenic causes chromosomal damage enabling them to participate in cell division and restrict in growth of cells. During the tenure of six months from 21st July to 20th October 2018, I worked very hard. The arsenic reduction in the grain was studied. Identification of gene single major gene conferring tolerance to arsenate responsible for arsenic resistance in the rice crop was located in rice genome. After Screening of 108 rice cultivars including a few photo-insensitive, were analyzed for arsenic accumulation ability in straw and grain with and without hull. straw accumulation was found significantly higher than grain. Short and long grain aromatic rice accumulates lesser amount of of arsenic than the high yielding cultivars four cultivars were considered as the donor parent for breeding new genotypes with low As- accumulation in brown rice. Less variability between phenotypic and genotypic coefficient of variation coupled with high heritability and genetic advance suggested the influence of

additive gene effect on the expression of gene responsible. Pot experiment and mapped the chromosomal region responsible for accumulation. Arsenite remain as the predominant inorganic form found in xylem sap of rice plants fed with either arsenate or arsenite. Four cultivars were selected and are used in breeding programs and list of candidate genes on chromosome 6 mapped between markers RZ516 AND RG213. This gene is used for physiological and molecular mechanism of Arsenic uptake. Bio remediation work was also performed for arsenic control in rice plants with micorrhizal fungi. and one paper has been given for publication. For Breeding of rice variety that has low grain arsenic levels or with a grain arsenic being present as less toxic. Identification of the chromosomal region responsible for grain and straw arsenic accumulation 101 recombinant inbred lines (RILs) along with their parents were utilized for phenotypic of two successive years 2015-2017 using AAS followed by genotyping using thirty nine polymorphic SSR. Four SSR were found to be located on chromosome 1, 2, 8 and 12 as observed from single point ANOVA Analysis. Likewise SSRs linked with arsenic accumulation in straw were located on chromosome 1, 4 and 6

Conclusion And Future Scope:-

Plants are made tolerant to Arsenic, or Arsenic are either made inactive or removed from underground water before they are absorbed by plants so that we can get arsenic free water and crop for our daily food. The arsenic removed (residue) can be used for plants as fertilizer for better development of plants. This technique are being used in few places in India this process is better than the process we used as salicylic acid where Arsenate treatment significantly enhanced total Fe accumulation in comparison to control plants. However, the most of the accumulated Fe was localized in the roots. It is evident from present work that SA has reduced the As^V induced oxidative stress and effectively modulated the enzymatic and non-enzymatic antioxidants. SA also played a role in enhancing Asc, GSH, and PCs in plants subjected to As^V stress. SA reduced the As accumulation in shoot and also overcame the As induced Fe deficiency in shoot. Based on the findings of the present investigation, the following future scope of studies will be undertaken to carry forward the research further. Microwave assisted extraction studies for arsenic, water hyacinths with ICP-AES method which will ensure the recovery of the metal ions To study the bioaccumulation kinetics and toxic effects of As. The aim of this project is to give an overview of the arsenic contaminant in rice plants and also the mechanism of mitigation of these toxic metals by different method with application of organic sources like green manure, FYM, BGA, and Azolla in integrated manner technique produces quality rice grain and more important ecologically and economically favourable yield and as it helps in reduction of arsenic uptake. Arsenic poisoning is due to repeated exposure to arsenic compounds like AS III and ASV. Improved technique which helps in reduction of arsenic uptake and produces quality rice grain and more important ecologically and economically favourable yield if followed then Even a 10% reduction in rice grain arsenic could save hundreds of thousands of lives caused due to health problems

Acknowledgments:-

Author is thankful to ugc govt. Of india for funding this post doctoral project on arsenic in rice. The author acknowledge principal and head of the department guru nanak institute of technology, 157/f, nilgunj road, panihati, sodepur, kolkata, west bengal 7001114, india, department of food technology, affiliated to Maulana Abul Kalam Azad University of Technology approved by AICTE, accredited by NAAC & NB India for providing facilities, and helping to complete this project smoothly. Also thankful to Kakali Madam, Anju Pal Madam, Suresh and Vardan.

References:-

1. Arnon D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. **Plant Physiol.** 24 1–15 10.1104/pp.24.1.1 [PMC free article] [PubMed] [Cross Ref]
2. Chattopadhyay T, Biswas T, Chatterjee M, Mandal N, S. Bhattacharyya S. 2008, Biochemical and SSR marker based characterization of some Bengal landraces of rice suffixed with 'Sail' in their name, *Ind. J. Genet. Pl. Breed.* 68: 15-20.
3. Dwivedi S, Tripathi RD, Srivastava S, Singh R, Kumar A, Tripathi P, Dave R, Rai UN, Chakrabarty D, Trivedi PK, Tuli R, Adhikari B, M. K. Bag MK. 2010, Arsenic affects mineral nutrients in grains of various Indian rice (*Oryza sativa* L.) genotypes grown on arsenic-contaminated soils of West Bengal, *Protoplasma.* 245: 113-124.
4. Mandal, Badal Kumar; Suzuki, K. T. (2012). "Arsenic round the world: a review". *Talanta* 58 (1): 201–235. doi:10.1016/S0039-9140(02)00268-0. PMID 18968746.
5. Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M. 2006, A silicon transporter in rice, *Nature.* 440: 688–691.

6. Peryea, F. J. (20–26 August 1998). "Historical use of arsenate insecticides, in soil contamination and implications for soil remediation" 16th World Congress of Soil Science. Montpellier, France Maximum accumulation was observed at harvest and the relative proportions accumulation in deferent plant parts appeared in the order: root > straw > grain . Liu et al. also corroborate such finding where proportional accumulations remained at 1.17 to 4.15 mg kg⁻¹(straw), 0.56–1.35 mg kg⁻¹(husk) and 0.25 to 0.73 mg kg⁻¹(grain) of rice.
7. Takahashi Y., Minamikawa R., Hattori K. H., Kurishima K., Kihou N., Yuita K. (2004). Arsenic behaviour in paddy fields during the cycle of flooded and non-flooded periods. **Environ. Sci. Technol.** 38:1038–1044 10.1021/es034383n [PubMed] [Cross Ref]
8. Tripathi P., Mishra A., Dwivedi S., Chakrabarty D., Trivedi P. K., Singh R. P. (2012). Differential response of oxidative stress and thiol metabolism in contrasting rice genotypes for arsenic tolerance. **Ecotoxicol. Environ. Saf.** 79: 189–198 10.1016/j.ecoenv.2011.12.019 [PubMed] [Cross Ref]
9. Wissuwa M, Yano M, Ae N. 1998 Mapping of QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.), *Theo. Appl. Genet.*, 97: 777-783.