



# **Effect of Arsenic Toxicity on Chlorophyll Content and Antioxidative Enzymes Activities in Rice Seedlings**

**Dinkar J. Gaikwad<sup>1\*</sup>, Apurba Pal<sup>2</sup> and Subhasis Mondal<sup>3</sup>**

<sup>1</sup>*Department of Biochemistry and Plant Physiology, Centurion University of Technology and Management, Odisha- 761211, India.*

<sup>2</sup>*Department of Basic Science (Crop Physiology), Horticulture College, Birsa Agricultural University, Ranchi, Jharkhand- 834006, India.*

<sup>3</sup>*Department of Plant Physiology, BCKV, Mohanpur, West Bengal-741252, India.*

## **Authors' Contributions**

*This work was carried out in collaboration among all authors. Author DJG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AP managed the literature searches. Author SM managed the analysis of the study. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/IJECC/2020/v10i1230318

### Editor(s):

(1) Dr. Gamal Abdel-Hafez Mahmoud Bekhet, King Faisal University, Saudi Arabia.

### Reviewers:

(1) Lidia Quintana, Universidad Nacional de Itapúa, Paraguay.

(2) Alireza Allameh, Rice Research Institute of Iran, Iran.

(3) Deijanira Albuquerque, Federal University of Mato Grosso, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63951>

**Original Research Article**

**Received 25 October 2020**  
**Accepted 30 December 2020**  
**Published 31 December 2020**

## **ABSTRACT**

**Aims:** Arsenic (As) contamination in rice is at alarming level as majority of rice growing regions in India are As contaminated. Present investigation is designed to study the better understanding of the physiological and biochemical mechanisms in the amount of chlorophyll change and antioxidative enzymes activity under arsenic stress on rice variety IET-4786 (Shatabdi).

**Study Design:** Completely Randomized Design.

**Place and Duration of Study:** The experiment was carried out in the departmental laboratory of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal during the year 2017-18.

**Methodology:** Two classes of inorganic arsenic- As(V) in the form of Sodium arsenate

\*Corresponding author: E-mail: dinkargaikwad02@gmail.com;

( $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ , M. W. = 321.01) and As(III) in the form of Sodium arsenite ( $\text{NaAsO}_2$ , M.W. =129.91) were added to the modified Hoagland nutrient solution@ 2.5, 5.0, 7.5, 10.0, 12.5,15.0  $\text{mg L}^{-1}$  concentration. After 20 days of treatment, rice seedlings under arsenate/arsenite treatments were analyzed for change in total chlorophyll content and antioxidative enzymes activity such as superoxide dismutase (SOD: EC 1.15.1.1), catalase (CAT: EC 1.11.1.6), ascorbate peroxidase (APX: EC1.11.1.11) and peroxidase (POD: EC 1.11.1.7).

**Results:** A noticeable decrease in total chlorophyll content was observed with arsenic treatments as compared to control. Various antioxidative enzymes showed significant variable response upon exposure to As(III) and As(V). SOD activities increased at low arsenic exposure up to 7.5  $\text{mg L}^{-1}$  but decreased with further increase in arsenic treatments. APX and POD activities were increased with increase in arsenic concentrations while CAT activity displayed decreasing trend.

**Conclusion:** The results are suggestive of differential metabolism of As(III) and As(V) in rice and could exert harmfulness in the early development stage of rice at inappropriate concentrations.

**Keywords:** Antioxidative enzymes; arsenic; stress; catalase; chlorophyll; peroxidase; rice; super oxide dismutase.

## 1. INTRODUCTION

Arsenic (As), a metalloid commonly known as “king of poisons” or “inheritance dust” and its lethal potential has been known for millennia that can undergo different ranges of biochemical and physiological interactions in plants. Since the last three decades, various natural and anthropogenic activities led to significant abiotic threats of Arsenic towards sustainable agriculture and human health through different mechanisms of toxicity. Soil type and plant species affect greatly the rate of uptake or accumulation of Arsenic (As). The problem of As has two aspects, it's a menace to sustainable crop production by creating stress as well as human health hazard due to incorporation of As in the food chain [1,2,3]. Arsenic is known to induce toxic reactive oxygen species (ROS) which are generated in the cell wall region as well as inside the cell during the process, which affects electron transport chain, membrane permeability, enzyme activity, metabolic pool, ion homeostasis that leads to decrease in plant biomass, leaf chlorosis and necrosis. Usually, plant roots are first tissue that exposed to As, that inhibits root extension and proliferation as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production [4-9]. Between the two inorganic forms, the highly oxidized pentavalent arsenate (As-V) is prevalent in the aerobic environment, while the highly reduced trivalent arsenite (As-III) is the predominant form in an anaerobic environment. Arsenate reduced to arsenite in plant tissue, does not normally have enough cytoplasmic concentrations to exert toxicity. Arsenite reacts with—SH group of

enzymes and proteins due to suppression of cellular function and death.

Rice (*Oryza sativa* L.) belongs to the so-called group of C3 grasses, is the staple food as well as main agricultural crop in India. India's role of being the second largest producer as well as consumer of rice besides the rice-based products is significant in the global economy. All activities of rice cultivation such as germination, raising of seedlings, transplanting and growing rice in main field are mostly done by groundwater irrigation. Rice production in India prefers two irrigation processes; in winter application of groundwater and in monsoon, rain-fed cultivation is preferred. But the presence of As in soil-water system hinders the rice plant inter-structural growth leading to the excess production of stress-responsive mechanism in rice plant. Generally, the As containing groundwater used as irrigation in paddy fields through shallow wells in India mainly in Ganges-Meghan-Brahmaputra basin [10,11] 3-4 cm standing water in the field leads to the decrease in soil pH and some soil microflora used to transform As into to more phytoavailable forms to rice plants [12-14]. Rice is an efficient crop in As uptake in comparison to other cereal crops [15,16]. Studies showed that As concentrations in rice plant depends on As presence in soil and/or irrigated ground water in addition to other factors governing the As mobility and uptake in plant-rhizosphere. In rice, As is taken up by plant roots using macro-nutrient transporters; arsenate via the phosphate while arsenite through Si transporters [17]. Rice cultivation in high As polluted agro-ecosystems resulted in inhibition in germination and seedling growth [18,19]; decrease in tillering [20,21]; reduction in shoot and root growth [22,23];

reducing yield at harvesting [24,25] and sometimes, leads to death [26]. Beside these, reduction in chlorophyll content in both seedling and flowering stages and enhanced As content in rice grain were also noted [27-29]. At cellular level, As not only damages chloroplast membrane that alters the functions of photosynthetic systems, but also serve as the site for the ROS generation. These ROS species are produced either by oxygen reduction in Mahler reaction or from chlorophyll due to the direct transfer of energy leading to the formation of atomic oxygen. Moreover, mitochondria, protein oxidation, and various metabolic pathways in peroxisomes also contribute in oxidative stress phenomenon in plants. As a result, the imbalance between ROS production and antioxidative system response is the direct cause of oxidative stress [30]. Plants have innate and efficient defense system to counter attack the toxic effects of As that penetrate into the cytosol. To protect from the sudden increase in intracellular levels of ROS, plant cells contain both enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), Guaiacol peroxidase (GPOX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) as well as nonenzymatic antioxidants, such as ascorbate, glutathione, carotenoid,  $\alpha$ -tocopherol and accumulation of anthocyanin in the leaves have been found to be very important [31, 32, 33]. Therefore, present study was conducted to compare the effect of two form of arsenic viz. As-V and As-III on chlorophyll content in relation with the change in antioxidative enzyme pattern.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material, Growth and Treatment Conditions

The experiment was conducted in laboratory condition under Bidhan Chandra Krishi Viswavidyalaya, Mohanpur. The rice genotypes IET-4786 (Shatabdi) was taken under study. The rice seedlings were grown on sand moistened with arsenate and arsenite solution under ambient laboratory condition. Two species of inorganic arsenic- As-V in the form of Sodium arsenate ( $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ , M. W.= 321.01) and As-III in the form of Sodium arsenite ( $\text{NaAsO}_2$ , M.W.= 129.91) were added to the modified Hoagland nutrient solution @ 2.5, 5.0, 7.5, 10.0, 12.5, 15.0  $\text{mg L}^{-1}$  concentration. After 20 days of treatment, rice seedlings under

arsenate/arsenite treatment were analyzed for change in total chlorophyll content and antioxidative enzyme activity such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD).

### 2.2 Enzyme Assays

The total chlorophyll contents in the leaves of 20 days old rice seedlings were calculated out by applying the Arnon [34] formulae *i.e.* Total Chlorophyll:  $20.2(A_{645}) + 8.02(A_{663})$ .

SOD (EC 1.15.1.1) activity was determined by the nitro-blue tetrazolium (NBT) photochemical assay method as described by Giannopolitis and Ries [35]. The reaction mixture (3 mL) contains 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75  $\mu\text{M}$  NBT, 0.1 mM EDTA, 2  $\mu\text{M}$  riboflavin and 0.1 mL of enzyme extract. The absorbance of the solution was measured at 560 nm in a UV-Vis spectrophotometer. SOD activity was expressed as Enzyme unit/milligram protein. One unit of SOD was defined as the amount of protein causing a 50% NBT photoreduction.

Total CAT (EC 1.11.1.6) activity was measured according the method of Beers and Sizer [36], with minor modifications. CAT was extracted in 50 mM K-phosphate buffer (pH 7.0) and 0.5% PVP-10, and its activity was assayed by measuring the reduction of  $\text{H}_2\text{O}_2$  at 240 nm ( $\epsilon=39.4/\text{Mcm}$ ) for 1 min.

APX (EC 1.11.1.11) activity was assayed following the method of Nakano and Asada [37]. The reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM  $\text{H}_2\text{O}_2$  and 0.1 mL enzyme extracts. The  $\text{H}_2\text{O}_2$ -dependent oxidation of ascorbate (AsA) was followed by a decrease in the absorbance at 290 nm with extinction constant 2.8/Mmcm. APX activity was expressed as  $\mu\text{mol}/\text{min}^{-1}\text{mg}^{-1}$  protein.

POD (EC 1.11.1.7) activity was assayed as per the modified method of summer and Gjessing [38], Mixture of 1 ml of o-dianisidine, 0.5 ml of  $\text{H}_2\text{O}_2$ , 1ml of phosphate buffer and 2.4ml of distilled water in the test tube, incubated at 30°C, Reaction was started by addition of 0.2ml of enzyme extract and stopped after 5min by adding 1ml of 2N  $\text{H}_2\text{SO}_4$ . Absorbance was recorded at 430nm. Expressed the activity of enzyme as change in absorbance/ min/g.

**Table 1. Effect of different concentrations of arsenate and arsenite on the activity on total chlorophyll content**

Treatments	As-III	As-V	Mean B
T1	1.317	1.317	1.317
T2	1.201	1.224	1.213
T3	1.088	1.125	1.107
T4	0.903	0.995	0.949
T5	0.785	0.885	0.835
T6	0.636	0.802	0.719
T7	0.504	0.727	0.615
<b>Mean A →</b>	0.919	1.011	
<b>Factors</b>	<b>(A)</b>	<b>(B)</b>	<b>(A X B)</b>
CD (p=0.05)	0.012	0.023	0.033
SE(m)±	0.004	0.008	0.011

\*A- arsenic treatments, \*\*B- arsenic concentrations

**Table 2. Effect of different concentrations of arsenate and arsenite on the activity of enzyme superoxide dismutase SOD activity (unit mg<sup>-1</sup> protein)**

Treatments	As-III	As-V	Mean B
T1	0.682	0.682	0.682
T2	1.750	1.114	1.432
T3	1.905	1.692	1.798
T4	2.522	1.987	2.254
T5	2.001	1.775	1.888
T6	1.623	1.522	1.573
T7	1.201	1.038	1.120
<b>Mean A →</b>	1.669	1.401	
<b>Factors</b>	<b>(A)</b>	<b>(B)</b>	<b>(A X B)</b>
CD (p=0.05)	0.023	0.044	0.062
SE(m)±	0.008	0.015	0.021

\*A- arsenic treatments, \*\*B- arsenic concentrations

**Table 3. Effect of different concentrations of arsenate and arsenite on the activity of enzyme catalase (μ mole of H<sub>2</sub>O<sub>2</sub> used/min/g weight)**

Treatments	As-III	As-V	Mean B
T1	0.805	0.805	0.805
T2	0.680	0.697	0.689
T3	0.505	0.579	0.542
T4	0.378	0.421	0.400
T5	0.258	0.323	0.291
T6	0.191	0.271	0.231
T7	0.125	0.217	0.171
<b>Mean A →</b>	0.420	0.473	
<b>Factors</b>	<b>(A)</b>	<b>(B)</b>	<b>(A X B)</b>
CD (p=0.05)	0.007	0.013	0.018
SE(m)±	0.002	0.004	0.006

\*A- arsenic treatments, \*\*B- arsenic concentrations

**Table 4. Effect of different concentrations of arsenate and arsenite on the activity of enzyme ascorbate peroxidase apx ( $\mu \text{ mol}/\text{min}^{-1}\text{mg}^{-1}$  protein)**

Treatments	As-III	As-V	Mean B
T1	1.97	1.97	1.965
T2	5.35	3.93	4.641
T3	8.03	5.90	6.962
T4	10.70	7.86	9.283
T5	14.38	9.83	12.104
T6	17.06	11.79	14.424
T7	23.41	15.72	19.566
<b>Mean A</b> →	11.556	8.142	
<b>Factors</b>	<b>(A)</b>	<b>(B)</b>	<b>(A X B)</b>
CD (p=0.05)	0.201	0.376	0.532
SE(m)±	0.069	0.129	0.182

\*A- arsenic treatments, \*\*B- arsenic concentrations

**Table 5. Effect of different concentrations of arsenate and arsenite on the activity of enzyme peroxidase (absorbance/min/g.)**

Treatments	As-III	As-V	Mean B
T1	6.61	6.61	6.607
T2	9.56	8.76	9.160
T3	12.49	10.24	11.362
T4	14.71	11.19	12.952
T5	16.52	12.09	14.303
T6	16.83	12.46	14.647
T7	17.17	13.92	15.542
<b>Mean A</b> →	13.413	10.751	
<b>Factors</b>	<b>(A)</b>	<b>(B)</b>	<b>(A X B)</b>
CD (p=0.05)	0.190	0.355	0.501
SE(m)±	0.065	0.121	0.172

\*A- arsenic treatments, \*\*B- arsenic concentrations

### 2.3 Statistical Analysis

The data represent mean calculated from three replicates  $\pm$  standard error (S.E). Two-way ANOVA was employed to confirm the variability of data and validity of results with different rates of as addition.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Arsenic Toxicity on Chlorophyll Content in Rice Seedling

Arsenic availability in soil can disturb normal functioning of plant metabolism such as impeded nutrient absorption, the negative effect on photosynthetic apparatus, the disruption of plant water status, interaction with the functional groups of enzymes, consequently leading to

stunted growth and low crop productivity [39, 40]. Decreases in chlorophyll (Chl) content and / or decreased photosystem-II activity are some common effects of As toxicity [41]. The results of present study on total chlorophyll content are summarized in (Fig. 1). The total chlorophyll content in the rice leaves changed with presence of arsenite and arsenate concentrations as compared to control. Highest chlorophyll content was observed in the plants with no arsenic exposure but it started to decrease with increase in arsenic concentration. The highest reduction in total chlorophyll content was noticed in the plants treated with arsenic treatment  $15 \text{ mg L}^{-1}$  concentration. Higher arsenic interferes with chlorophyll synthesis and disrupts photosynthetic apparatus that consequently lowers effectiveness of PS-II [10].

### 3.2 Effect of Arsenic Toxicity on Antioxidant Enzyme Activity in Rice Seedling

Presence of arsenic in soil and water can create abiotic stress leading to overproduction of reactive oxygen species (ROS) that alter redox state of the cells [42, 43]. Plants have innate and efficient defense system to counter attack the toxic effects of As that penetrate into the cytosol. To protect from the sudden increase in intracellular levels of ROS, increasing activities of the antioxidant enzymes has been shown [44]. Activities of different antioxidant enzymes such as SOD, CAT, APX and POD significantly varied in all arsenic treated rice seedlings. Among these antioxidant enzymes, SOD is a key player that constitutes the first line of defense against ROS in plants. SOD belongs to a group of metalloenzymes and catalyzes the dismutation of superoxide free radicals ( $O_2^-$ ) into  $O_2$  and  $H_2O_2$ . Arsenic-induced increase in the activity of SOD can be due to an enhanced level of  $O_2^-$  or the direct action of As on SOD. In plants, As has been reported to either increase or decrease CAT activity [45, 46]. In this present experiment, The SOD activity of the rice seedlings under different arsenic treatments are shown in (Fig. 2). The SOD activities of the rice seedlings displayed increasing trend at lower arsenic exposure with maximum increase at  $7.5 \text{ mg L}^{-1}$ . Higher exposure to As-III and As-V caused decline in SOD activity. Plants exposed

to As-III shown significant increase in SOD in comparison to As-V. Higher activity of SOD may be the cause of this  $H_2O_2$  production during the arsenic stress. The variation in catalase activity (Fig. 3) of rice variety shatabdi due to different treatments of arsenic was statistically significant ( $p=0.05$ ) among the concentrations. The highest catalase activity under this treatment was observed in arsenic @ 0 ppm (control) and then decreasing trend was observed with increasing concentrations of arsenic. The mean catalase activity over different concentrations of arsenite was higher than that of arsenate indicating the latter form of arsenic as a stronger toxicant. According to CAT isozyme chart, CAT isozyme bands were strong with low concentrations of As, and it meant that the activity was enhanced. With the increase of As, CAT isozyme bands became dark, and it meant that the activity decreased, which coincided with the measured results of CAT activity. As a member of ascorbic acid-glutathione cycle, APX plays a crucial role in eliminating poisonous  $H_2O_2$  from plant cells. The APX activity was found to be higher at all concentrations in response to As-III and As-V exposed plants. There was significant increase in mean values of As-III over As-V treatments (Fig. 4). APX is a heme-containing protein located in plastid stroma and membrane. In the presence of APX and two molecules of ascorbate the hydrogen peroxide get reduced into water and two molecules of monohydroascorbate are also generated [47].

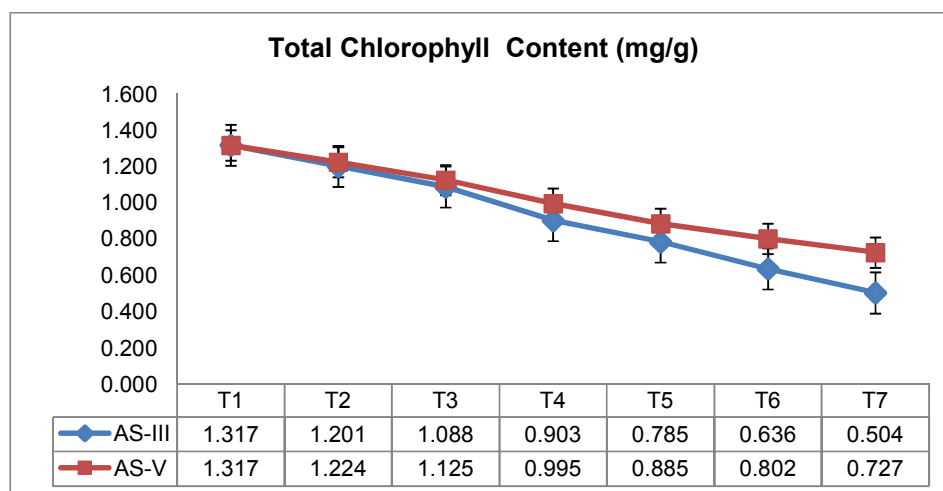
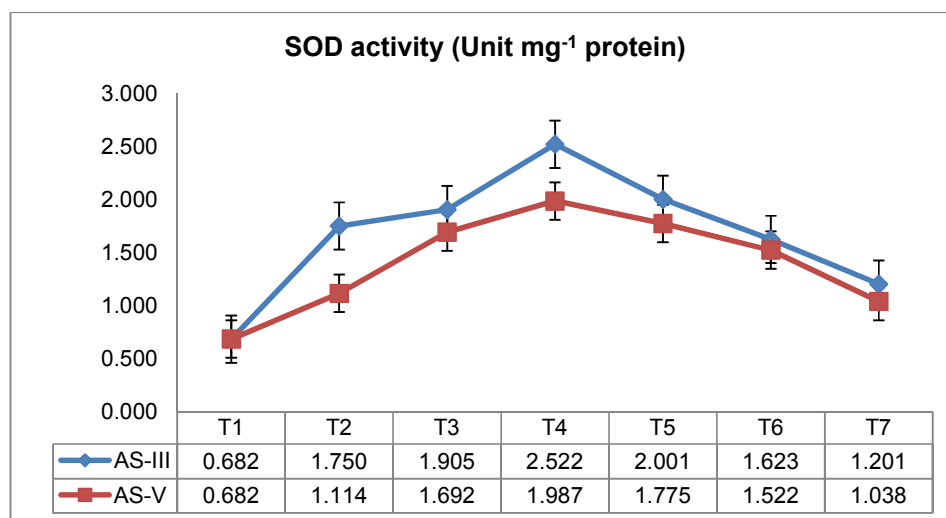
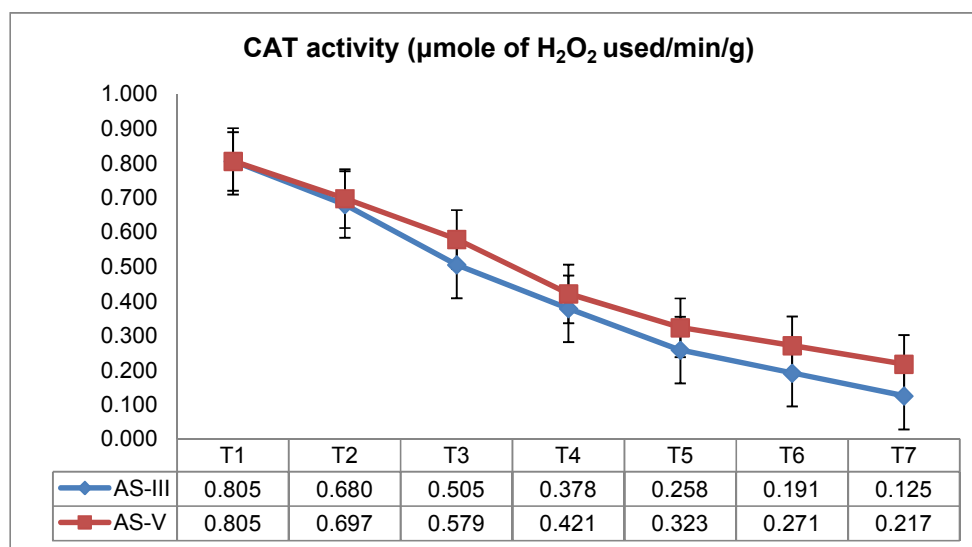


Fig. 1. Effect of different concentrations of arsenate and arsenite on the activity on total chlorophyll content



**Fig. 2. Effect of different concentrations of arsenate and arsenite on the activity of enzyme superoxide dismutase SOD activity (unit mg<sup>-1</sup> protein)**



**Fig. 3. Effect of different concentrations of arsenate and arsenite on the activity of enzyme catalase (µ mole of H<sub>2</sub>O<sub>2</sub> used/min/g weight)**

Upregulation of APX activity has been reported in rice seedling [48], Mung bean [49], Beans [50] and Maize [51] exposed to arsenic. The effects of arsenic toxicity on POD activity are presented in (Fig. 5). POD activity increased significantly with the increase of arsenic concentrations. Analysis of variance showed that POD activity and arsenic concentrations had significant effects ( $p=0.05$ ). Highest POD activities were observed in the arsenic 15 mg L<sup>-1</sup> concentration. According to POD isozyme chart, with the

increase of the concentration of As, POD isozyme bands color was stronger, and it meant that the activity was increased gradually, which coincided with the measured results of POD activity. Increased followed by decreased SOD activity coupled with the increased APX activity and low CAT level might be responsible for higher accumulation of H<sub>2</sub>O<sub>2</sub> in leaves of As-treated rice plants than control plants.

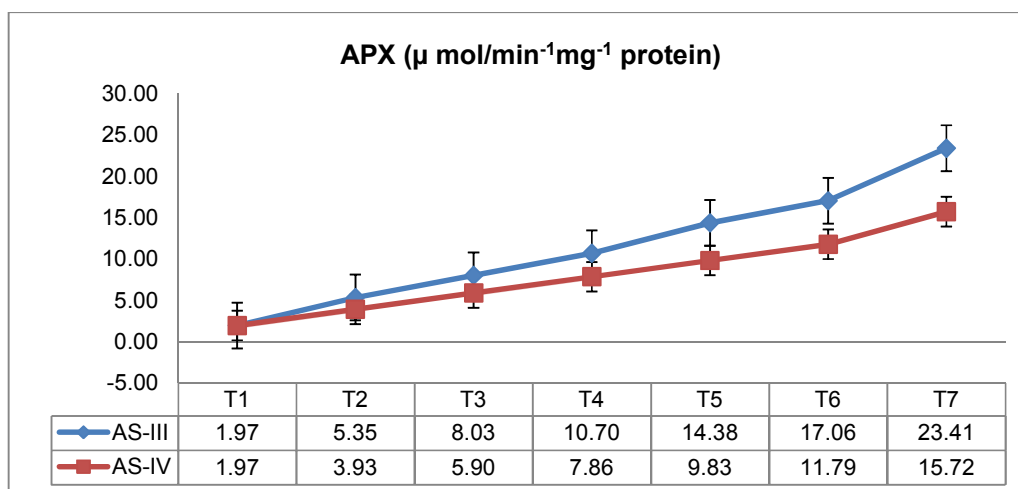


Fig. 4. Effect of different concentrations of arsenate and arsenite on the activity of enzyme ascorbate peroxidase APX ( $\mu \text{ mol/min}^{-1}\text{mg}^{-1} \text{ protein}$ )

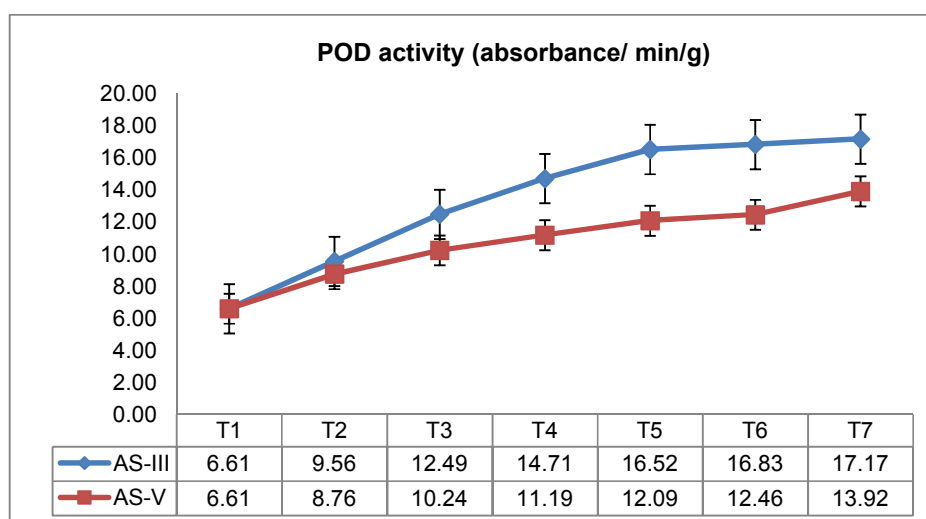


Fig. 5. Effect of different concentrations of arsenate and arsenite on the activity of enzyme peroxidase (absorbance/ min/g.)

#### 4. CONCLUSION

The results of this study suggest that, arsenic induced enhancement in the activities of antioxidant enzymes. The increase in APX, SOD, POD activities could represent an appropriate protection against overproduction of peroxides when As accumulated in rice. It may represent a fundamental defensive mechanism against oxidative stress which prevents biological damage mediated by ROS. We observed that rice crop is more sensitive to As-III than As-V toxicity and all data demonstrate

that As-III is more toxic to these plants than As-V.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Meharg AA, Williams PN, Adomako E, Lawgali YY, Deacon C, Villada A. et al. Geographical variation in total and inorganic arsenic content of polished



- (white) rice. J Environ Sci Technol. 2009;43(5):1612-7.
2. Hasanuzzaman M, Roychowdhury R, Karmakar J, Dey N, Nahar K, Fujita M. Recent advances in biotechnology and genomic approaches for abiotic stress tolerance in crop plants; 2015. DOI: 10.1201/b18597-14
  3. Singh NK, Raghubanshi AS, Upadhyay AK, Rai UN. Arsenic and other heavy metal accumulation in plants and algae growing naturally in contaminated area of West Bengal, India. Ecotoxicol Environ Saf. 2016;130:224–232.
  4. Han B, Runnells T, Zimbron J, Wickramasinghe R. Arsenic removal from drinking water by flocculation and microfiltration. Desalination. 2002;145: 293-298.
  5. Garg N, Singla P. Arsenic toxicity in crop plants: Physiological effects and tolerance mechanisms Environ Chem Lett. 9. 2011;303–321. DOI: 10.1007/s10311-011-0313-7
  6. Talukdar D. Arsenic-induced oxidative stress in the common bean legume, *Phaseolus vulgaris* L. seedlings and its amelioration by exogenous nitric oxide. Physiol Mol Biol Plants. 2013;19(1):69–79.
  7. Chandrakar V, Dubey A, and Keshavkant S. Modulation of antioxidant enzymes by salicylic acid in arsenic exposed *Glycine max* L.J Soil Sci Plant Nutri. 2016;16(3):662-676.
  8. Pandey C, Khan E, Panthri M, Tripathi RD, Gupta M. Impact of silicon on Indian mustard (*Brassica juncea* L.) root traits by regulating growth parameters, cellular antioxidants and stress modulators under arsenic stress. Plant Physiol Biochem. 2017;104:216–225.
  9. Bakhat HF, Zia Z, Fahad S, Abbas S, Hammad HM, Shahzad AN, et al. Arsenic uptake, accumulation and toxicity in rice plants. Possible remedies for its detoxification a review. Environ Sci Pollut Res. 2017;24:9142–9158.
  10. Anjum SA, Tanveer M, Hussain S, Shahzad B, Ashraf U, Fahad S, et al. Osmoregulation and antioxidant production in maize under combined cadmium and arsenic stress. Environ Sci Pollut Res. 2016;23(12):11864–11875.
  11. Meharg AA, Zhao FJ. Arsenic, Rice. Netherlands: Springer Science & Business Media. 2012. DOI:10.1007/978-94-007-2947-6
  12. Islam MR, Jahiruddin M, Islam S. Assessment of arsenic in the water-soil-plant systems in Gangetic floodplains of Bangladesh. Asian Journal of Plant Sciences. 2004;3:489-493.
  13. Heikens A, Panaullah GM, Meharg AA. Arsenic behaviour from groundwater and soil to crops: Impacts on agriculture and food safety. Rev Environ Contam Toxicol. 2007;189:43–87.
  14. Heikens A. Arsenic Contamination of Irrigation Water, Soil and Crops in Bangladesh: Risk implications for Sustainable Agriculture and Food safety in Asia. Food and Agricultural Organization of the United Nations Regional Office for Asia and the Pacific; 2006.
  15. Bhattacharya P, Samal AC, Majumdar J, Santra SC. Accumulation of arsenic and its distribution in rice plant (*Oryza sativa* L.) in Gangetic West Bengal, India. Paddy and Water Environment. 2010;8(1):63-70.
  16. Su Y H, McGrath S P, Zhao F J. Rice is more efficient in arsenite uptake and translocation than wheat and barley. Plant Soil. 2010;328:27–34. DOI: 10.1007/s11104-009-0074-2
  17. Ma JF, Yamaji N, Mitani N, Xu XY, Su YH, McGrath SP, et al. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Natl Acad Sci. U.S.A. 2008;105:9931–9935. DOI: 10.1073/pnas.0802361105
  18. Jahan I, Hoque S, Ullah SM, Kibria MG. Effects of arsenic on some growth parameters of rice plant. Dhaka Univ J Biol Sci. 2003;12:71- 77.
  19. Moulick D, Santra SC, Ghosh D. Seed priming with Se alleviate as induced phytotoxicity during germination and seedling growth by restricting as translocation in rice (*Oryza sativa* L c.v. *IET-4094*). Ecotoxicology and Environmental Safety. 2017;145:449-456.
  20. Rahman MA, Hasegawa H, Rahman MM, Islam MN, Miah MM, Tasmien A. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. Chemosphere. 2007;67:1072–1079. DOI:10.1016/j.chemosphere.2006.11.061.
  21. Rahman MA, Rahman MM, Majid Miah MA, Khaled HM. Influence of Soil Arsenic Concentrations on Rice (*Oryza sativa* L.) Subtrop Agric Res & Dev. 2004;2(3):24-31.

22. Cox MS, Bell PF, Kovar JL. Different tolerance of canola to arsenic when grown hydroponically or in soil. *J Plant Nutri.* 1996;19:1599-1610.
23. Abedin MJ, Meharg AA. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). *Plant and Soil.* 2002;243:57-66.
24. Khan MA, Islam MR, Panaullah GM, Duxbury JM, Jahiruddin M, Loeppert RH. Accumulation of arsenic in soil and rice under wetland condition in Bangladesh. *Plant Soil.* 2010;333:263-74.
25. Moulick D, Chowardhara B, Panda SK. Agroecotoxicological aspect of arsenic (As) and cadmium (Cd) on Field crops and its mitigation: Current status and future prospect. In *Plant-metal Interactions* Springer Cham. 2019:217-246.
26. Marin AR, Masscheleyn PH, Patrick WH Jr. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. *Plant Soil.* 1992;139:175-183.
27. Moulick D, Santra SC, Ghosh D. Effect of selenium induced seed priming on arsenic accumulation in rice plant and subsequent transmission in human food chain. *Ecotoxicol Environ Saf.* 2018;152:67-77.
28. Moulick D, Santra SC, Ghosh D. Rice seed priming with se: A novel approach to mitigate as induced adverse consequences on growth, yield and as load in brown rice. *J Hazard Mater.* 2018;355:187-196.
29. Moulick D, Santra SC, Ghosh D. Seed priming with se mitigates as-induced phytotoxicity in rice seedlings by enhancing essential micronutrient uptake and translocation and reducing as translocation. *Environ Sci Pollut Res.* 2018;25(27):26978-26991.
30. Sahay S, Gupta M. An update on nitric oxide and its benign role in plant responses under metal stress. *Nitric Oxide.* 2017;67:39-52.
31. Yadu B, Chandrakar V, Keshavkant S. Glycinebetaine reduces oxidative injury and enhances fluoride stress tolerance via improving antioxidant enzymes, proline and genomic template stability in *Cajanus cajan* L. *South Afr J Bot.* 2017;111:68-75.
32. Yadu B, Chandrakar V, Keshavkant S. Responses of plants towards fluoride: An overview of oxidative stress and defense mechanisms. *Fluoride.* 2016; 49:293-302.
33. Xalxo R, Yadu B, Chakraborty P, Chandrakar V, Keshavkant S. Modulation of nickel toxicity by glycinebetaine and aspirin in *Pennisetum typhoideum*. *Acta Biol Szeged.* 2017; 61:163-171.
34. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris* *Plant Physiology.* 1949;24:1-15.
35. Giannopolitis CN, Ries SK. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 1977;59(2):309-314.
36. Beers RF, Sizer W Jr. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem.* 1952;195:133-140.
37. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 1981;22:867-880.
38. Summer JB, Gjessing EC. Determination of peroxidase activity. *Arch Biochem.* 1943; 2:291.
39. Armendariz AL, Talano MA, Villasuso AL, Travaglia C, Racagni GE, Reinoso H, et al. Arsenic stress induces changes in lipid signalling and evokes the stomata closure in soybean. *Plant Physiol Biochem.* 2016;103:45-52.
40. Mehmood T, Bibi I, Shahid M, Niazi NK, Murtaza B, Wang H, et al. Effect of compost addition on arsenic uptake, morphological and physiological attributes of maize plants grown in contrasting soils. *J Geochem Explor.* 2017;178:83-91.
41. Duman F, Ozturk F, Aydin Z. Biological responses of duckweed (*Lemna minor* L.) exposed to the inorganic arsenic species As(III) and As(V): Effects of concentration and duration of exposure. *Ecotoxicol.* 2010;19:983-993.
42. Sharma I. Arsenic induced oxidative stress in plants. *Biologia.* 2012;67(3):447-453.
43. Finnegan PM, Chen W. Arsenic toxicity: The effects on plant metabolism *Front Physiol.* 2012;3:1-18.
44. Souri Z, Karimi N, de Oliveira LM. Antioxidant enzymes responses in shoots of arsenic hyperaccumulator, *Isatis cappadocica* Desv, under interaction of arsenate and phosphate. *Environ. Technol.* 2017;1-32.
45. Gusman GS, Oliveira JA, Farnese FS, Cambraia J. Mineral nutrition and enzymatic adaptation induced by arsenate and arsenite exposure in lettuce

- plants. *Plant Physiol Biochem.* 2013; 71:307–314.
46. Abbas G, Saqib M, Akhtar J, Murtaza G, Shahid M. Effect of salinity on rhizosphere acidification and antioxidant activity of two acacia species. *Can J for Res.* 2014;45:124–129.
47. Noctor G, Foyer CH. Ascorbate and glutathione: Keeping active oxygen under control *Ann Rev Plant Physiol.* 1998;49: 249–279.
48. Shri M, Kumar S, Chakrabarty D, Trivedi PK, Malick S, Mishra P, et al. Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedling. *Ecotoxicol Environ Safety.* 2009;72:1102–1110.
49. Singh HP, Batish DR, Kohali RK, Arora K. Arsenic induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regul.* 2007; 53:65–73.
50. Stoeva N, Berova M, Vassilev A, Zlatev Z. Effect of exogenous polyamine diethylenetriamine on oxidative changes and photosynthesis in As-treated maize plants (*Zea mays* L.). *J Cent Eur Agric.* 2005;6:367–374.
51. Miteva E, Peycheva S. Arsenic accumulation and effect on peroxidase activity in green bean and tomatoes Bulg. *J Agric Sci.* 1999;5:737–740.

© 2020 Gaikwad et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle4.com/review-history/63951>