Original Research Article

Variations in oxidative and antioxidant responses of rice plant to arsenic contaminated areas of Assam.

ABSTRACT

Rice is a common food in the state of Assam, and because it is grown in arsenic contaminated soil, eating it might expose people to arsenic. Standard procedures were used while analysing arsenic content in various parts of rice plant and other biochemical indicators related to arsenic stress. In descending order, the rice plant's parts that accumulate arsenic are root, straw, leaf, husk, and grain.With a reduction in the build-up of arsenic, it was observed that the catalase activity increased, although the ascorbic acid oxidase activity, hydrogen peroxide content, malondialdehyde content, and proline content all decreased. It is clear from the current study that the amount of arsenic in the rice grain (0.094 to 0.147 ppm) is below the permissible limit prescribed by FAO/WHO, but since the local population typically eats rice three times a day, which may cause bioaccumulation so efforts can be made to check arsenic in the cooked rice as well.

Keywords: *Antioxidant enzyme, Arsenic, Biochemical, Oxidative stress marker*

1. INTRODUCTION

There is a lot of arsenic (As) in the environment, and several countries, including India, have found it in their soil and water. In aerobic soils, as is primarily present as arsenate in an oxidized state (AsV).The World Health Organization and the U.S. Environmental Protection Agency's acceptable limit of 0.01 ppm for arsenic in groundwater has been exceeded in India, notably in the north-eastern region [1]. Arsenic pollution of crops and vegetables also negatively impacts human health in addition to contaminating groundwater. Arsenic in groundwater harm the worldwide population's health in more than 50 countries. Rokonuzzaman *et al.* [2] outlines the risks to human health associated with eating As-contaminated rice, evaluates various biomarkers for determining As levels in the body, and discusses the most recent developments in As-reducing technologies, with a focus on the use of seed priming, nanotechnology, and biochar to reduce As levels in rice grains.

Rice grains accumulate arsenic more efficiently than other cereal crops. This is owing to the fact that rice is frequently farmed in wet soil, where a more inorganic mobile form (AsIII) predominates, resulting in reduced conditions [3]. It causes a variety of physio-chemical changes in plant tissue as well as changes in redox potential, resulting in the creation of reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide, and others, causing oxidative stress and disrupting numerous metabolic activities within the cell [4]. To counteract oxidative stress and minimise ROS production, plants contain an antioxidative defence system that comprises enzymes like ascorbic acid oxidase (AOX), catalase (CAT), and others [5].

All arsenic species can travel through the plant cell via specialised transporter proteins [6], altering physiological processes and cellular response, resulting in growth and development suppression. In the north-eastern region of India, primarily in Assam, rice is the most important cereal food crop grown using the traditional method under submerged conditions, with AsIII being the most common kind. The total arsenic accumulation in soil varies from 5.7 to 12.4 ppm [7], while the total arsenic accumulation in grains varies from 0.9 to 1.15 ppm, both of which are more than the permitted limit [8], according to research done in Assam (Barak valley).In light of the aforementioned, the current study looked at various biochemical markers related to oxidative stress brought on by arsenic build-up and rice plant parts grown in Assam's arsenic-contaminated areas.

2. MATERIALS AND METHODS

The rice plant, the Ranjit mega variety (high yielding, semi dwarf, short, fine grain quality), as well as a soil sample, were gathered from two distinct sites in the Jorhat region, namely Nagajanka and Titabar, which are reported to be arsenic contaminated areas as per the works of Saikia *et al*. [9]. In addition, a recent study conducted by the Assam Public Health Engineering Department (APHED), UNICEF, and School of Environmental Studies (SoES), Jadavur University, India, in the Assam districts of Jorhat and Golaghat, showed groundwater arsenic pollution.

The samples were taken at the time of harvest, and the levels of various biochemical parameters, including arsenic and the contents of hydrogen peroxide, malondialdehyde, proline, catalase activity, and ascorbic acid oxidase in various parts of the rice plant, were estimated. Using the approach outlined by Mclaren *et al.* [10] the total amount of arsenic in soil and rice plant sections was determined. The procedures outlined by Velikova *et al*. [11], Hodges *et al*. [12], Bates *et al*. [13], Beers and Sizer [14], were used for determination of hydrogen peroxide content, malondialdehyde content, proline content, catalase activity respectively. The procedure described by Drumm *et al*. [15] was used to measure ascorbic acid oxidase activity. The method outlined by Lowry *et al*. [16] was used to determine the enzyme extracts' total soluble protein concentration. IBM SPSS Statistics 21 was used to examine the data. To compare the mean values from three replications one way ANOVA was used, followed by the least significant difference test at a P-value of less than 5%.

3. RESULTS AND DISCUSSION

3.1 Arsenic content

Due to its regular ingestion through numerous food items, mostly rice and rice products, arsenic is considered to be one of the most toxic and carcinogenic substances and may have a significant detrimental impact on the human metabolic system. Because it is grown in anaerobic circumstances, rice collects 10 times more inorganic arsenic than any other cereal crop. For Nagajanka and Titabar, respectively, the soil samples' arsenic concentrations were found to be 3.524 ppm and 1.774 ppm (Table 1). As a result, it has been demonstrated that Nagajanka samples had higher arsenic deposition in a number of rice plant regions. Arsenic build-up occurred in the following descending order: Roots>Straw>Leaf>Husk>Grain which is also supported by the findings of earlier researchers [17]. It was also observed that the arsenic content in rice grains ranges from 0.094 to 0.147 ppm, which is below the permissible limit. However, it is important to note that the arsenic content in straw ranges from 0.495 to 0.645 ppm, which is above the permissible limit, raising additional concerns given that straw is used as cattle feed.

3.2 Hydrogen peroxide (H_2O_2) content

One of the earliest responses of plants to oxidative stress, both biotic and abiotic, is the production of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS). In the current investigation, it was found that when the amount of arsenic in the plant components grows, so does the H_2O_2 level. It was discovered that samples from the Nagajanka location had higher levels of arsenic buildup, which caused their H_2O_2 concentration to be higher (0.069-0.151 M/g fw), compared to those from the Titabar location, where it is relatively lower (0.055-0.122 M/g fw) (Table 1). Given that arsenic accumulation was seen in all the various portions of the rice plant in both locations and given that these findings are corroborated by the literatures [18, 19], this rise in H_2O_2 concentration may be the result of stress.

3.3 Malondialdehyde (MDA) content

A measure for oxidative stress and redox signalling is malondialdehyde (MDA) concentration. In the current investigation, samples taken from the Nagajanka location had much higher MDA content (0.255–3.311 M/g fw), while samples taken from the Titabar location had significantly lower MDA content (0.127–3.066 M/g fw) (Table 1). When MDA formation is activated in stress conduction, it is obvious that, as arsenic buildup decreases, so does the MDA level in the rice plant sections. Similar results were also obtained from investigations by Majumder *et al*. [19].

3.4 Proline content

Proline is one of the compatible solutes that plants under stress accumulate in significant amounts because it helps maintain the osmotic equilibrium inside cells, neutralise ROS, and protect membrane integrity. The proline in the rice samples from the Nagajanka site (9.172-95.592 g/g fw) was found to be greater than the samples from the Titabar location (7.666-82.309 g/g fw) in the current investigation (Table 1). This indicates that stress brought on by the presence of arsenic in the various rice plant parts causes the proline content to decrease with decreasing arsenic accumulation in the rice plant parts, which is also corroborated by previous literature [19, 20].

3.5 Catalase (CAT) activity

The ability of plants to adjust to changes in their surroundings is aided by reactive oxygen species. An important antioxidant enzyme that degrades H_2O_2 is catalase. In the current investigation, the roots (53.470-60.586 units/min/g fw) where the arsenic buildup was larger had the lowest catalase activity (0.851-1.655 ppm). A similar conclusion that the catalase activity will rise with a decline in arsenic accumulation can also be drawn from the study. The results of Majumder *et al*. [19] and Kumar *et al*. [21] concur with the findings of the current study. It is also important to note that the rise in hydrogen peroxide level in arsenic stress is caused by decreased catalase activity, which is consistent with the current findings.

3.6 Ascorbic Acid Oxidase (AOX) activity

Ascorbic acid oxidase activates to protect the plant cells if there is a change in the microenvironment brought on by oxidative stress. In the present study the AOX activity increases with the increase in the arsenic accumulation and the samples of Nagajanka (1.753-5.333 µmoles ascorbic acid disappeared/min/g fw) were found to have higher activity as the arsenic content was higher as compared to Titabar (1.493-4.482 µmoles ascorbic acid disappeared/min/g fw) (Table 1) samples as the arsenic status of the soil was low supported by the works from Ghosh and Biswas [22]. A decrease in AOX activity has been linked by some researchers to the hyperaccumulation of arsenate in the plant's various sections.

4. CONCLUSION

The Nagajanka area had the greatest level of arsenic in the soil sample (3.524 ppm), while Titabar had the lowest level (1.774 ppm). The arsenic level ranged from 0.851 to 1.655 ppm in roots and from 0.094-0.147 in grains. Grain, husk, leaf, straw, and root are in ascending order of arsenic accumulation. With an increase in arsenic accumulation, the ascorbic acid oxidase activity, hydrogen peroxide content, malondialdehyde content, and proline content increased while the catalase activity decreased. Arsenic in cooked rice can be a future venture to get a better insight on its retention.

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Table 1. Biochemical parameters in different parts of rice plant (Var. Ranjit) from two different locations of Jorhat district of Assam

Parameters	Location	Soil	Roots	Straw	Leaf	Husk	Grains	p<0.05	F Value
Arsenic content (mg/kg fw)	Nagajanka	3.524^{d}	1.655^{c}	0.645^{b}	0.640^{b}	0.213^{a}	0.147^{a}	0.0001	613.515
	Titabar	1.774^{d}	0.851^{c}	0.495^{b}	0.426^{b}	0.162^{a}	0.094^{a}	0.001	278.618
HydrogenPeroxide content (µM/g fw)	Nagajanka	-	0.151^{c}	0.112^{b}	0.083^{a,b}	0.073^{a}	0.069^{a}	0.0001	28.417
	Titabar	-	0.122^{c}	0.091^{b}	0.074^{a,b}	0.060^{a,b}	0.055^{a}	0.0001	19.535
Malondialdehyde content (µM/g fw)	Nagajanka	-	3.311^{e}	2.722^{d}	2.273^{c}	1.554^{b}	0.255^{a}	0.0001	6144.080
	Titabar	-	3.066^{e}	2.257^{d}	2.044^{c}	1.344^{b}	0.127^{a}	0.001	2660.021
Proline content (µg/g fw)	Nagajanka	-	95.592^{e}	82.984^{d}	32.979^{c}	22.647^{b}	9.172^{a}	0.0001	2634.760
	Titabar	-	82.309^{e}	62.668^{d}	36.651^{c}	23.036^{b}	7.666^{a}	0.0001	2225.744
Catalase Activity (units/min/g fw)	Nagajanka	-	53.470^{a}	64.050^{b}	65.483^{b}	71.420^{c}	86.253^{d}	0.0001	240.220
	Titabar	-	60.586^{a}	68.233^{b}	72.078^{c}	77.078^{d}	92.186^{e}	0.0001	1515.675
Ascorbic Acid Oxidase Activity (µmoles ascorbic acid disappeared /min/g fw)	Nagajanka	-	5.333^{d}	5.023^{c}	4.861^{c}	2.438^{b}	1.753^{a}	0.0001	769.221
	Titabar	-	4.482^{e}	4.331^{d}	3.857^{c}	1.975^{b}	1.493^{a}	0.0001	2979.643

[a-d]Mean in the same row with different letters are significantly different ($p<0.05$)