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Title of the Project

Screening of arsenic tolerant and sensitive rice (*Oryza sativa* L.) cultivars grown in the arsenic contaminated soil of West Bengal on the basis of tolerance, growth and metabolism

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Introduction

Increased concentrations of As severely affects plant growth by arresting biomass accumulation which can led to drop in productivity (Chandrakar et al. 2016a). Arsenic (As) present as two major species - arsenate (AsV) and arsenite (AsIII) of which AsIII is considered as more toxic than AsV (Hughes et al. 2011). Arsenate is taken up by plants which gets converted into arsenite by the activity of arsenate reductase. The combination of AsV and AsIII is designated as total arsenic (Kumar and Riyazuddin 2008; Abdel-Lateef et al. 2013). As triggers various physiological alterations within the plant tissue including changes in redox potential. Arsenic enters in plant-root system either as arsenate [As(V)] or arsenite [As(III)] of which latter one is considered as most harmful to all organisms. Nearly all plant species poses As(V) reduction ability through arsenate reductase (AR) enzyme, thereby, As(III) is found to be dominant species within the plant tissues under As(V) challenged environment (Dhankher et al. 2006; Xu et al. 2007). Arsenic accumulation interrupts prooxidant-antioxidant balance, instigates signal transduction and leads to formation of reactive oxygen species viz., superoxide radical ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) within the cell that eventually promotes oxidative stress (Leterrier et al. 2012; Hasanuzzaman and Fujita 2013; Nath et al. 2014). Balanced proportion of superoxide radical to H_2O_2 contents is the key regulator of programme cell death and respiratory cycle in mitochondria (Sabater and Martin 2013; Andronis et al. 2014). H_2O_2 is also known to play an essential role in Mitogen-activated protein kinase (MAPKs) mediated cellular responses under As stress (Huang et al. 2012). Disruption of redox homeostasis can causes intense damage to DNA, protein and lipid which leads to inhibition in regular cellular functioning (Srivastava et al. 2011; Chandrakar et al. 2017a). Membrane phospholipid (poly unsaturated fatty acid, PUFA) is the principal target of ROS and the extent of membrane damage by measuring the chief product of lipid peroxidation i.e MDA, is a way to estimate level of injury and As stress tolerance ability of plant as well (Rafiq et al. 2017). MDA production correlates with the inactivation of membrane proteins and its receptors that ultimately results into the leakage of electrolytes (Kaur et al. 2012; Yadu et al. 2016). Level of ROS generation varies widely with plant species and intensity of heavy metal stress (Hasanuzzaman et al. 2012b). Furthermore, under heavy metal induced toxicity, plants accumulate an osmoprotectant, proline to combat water stress and leads to enhancement of oxidative stress tolerance by quenching of free radicals (Verbruggen et al. 2009; Agami 2014; Fariduddin et al.

2015). Competent defence mechanism along with activities of enzymatic and non-enzymatic antioxidants are induced under As stress to protect plants from adversities of oxidative stress where initial steps taken towards ROS detoxification by superoxide dismutase (SOD), which catalyzes conversion of superoxide radical ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) that later on converted into water by the activity of catalase (CAT) enzyme (Hasanuzzaman et al. 2012a; Gusman et al. 2013b; Chandrakar et al. 2016a). It has been reported that antioxidant enzymes activities in different plant species alter differentially for physiological adaptation with respect to As stress due to substantial genetic variation (Hossain et al. 2012; Gill et al. 2014; Farooq et al. 2015a, 2015b).

Ascorbic acid (AA) and its related enzymes ascorbate peroxidase (APX) and ascorbate oxidase (AOX) provide antioxidant properties in plants against oxidative injury. According to Mazid et al. (2011), AA has the ability to amplify plant growth and development by its antioxidant property correlated with the resistance to oxidative damage. Glutathione is a low molecular mass tripeptide and nonenzymatic antioxidant thiol. It occurs in both reduced (GSH) and oxidized (GSSG) forms. Glutathione protects the plants against heavy metal toxicity, controls essential gene expression and provides resistance to various environmental stresses. The presence of higher level of GSSG than GSH is a sign of oxidative stress in plant cell. The capability of thiol groups of GSH to bind with As(III) favors the formation of As(III)-GSH conjugates and transported them from cytosol into vacuole. GSH stimulates the As detoxification process by altering the activity of glutathione-S-transferase (GST) that participates in the removal of toxic compounds by maintaining the reduced form of sulfhydryl (-SH) groups of cysteine and involves in major toxicity regulating pathway AA-GSH cycle (Singh et al. 2015). GST and glutathione peroxidase (GPx) both detoxify ROS and also enhance the conjugation of toxic molecules with GSH. According to Anderson and Davis (2004) GST, GPx and GR use GSH to play a crucial role in plant resistance. Therefore, the present work was aimed to ascertain the changes of GSH contents and activities of GSH-associated enzymes following the induction of As(V) toxicity in growing rice seedlings.

Phytochelatins (PCs) are heavy metal binding, cysteine rich polypeptides. Both As(III) and As(V) anions have been reported to stimulate the formation of PCs in plants (Grill et al. 2000). GSH is the precursor for PC synthesis that involves transpeptidation reaction catalyzed by PC synthase (Grill et al. 2007). PC synthesis is directly proportional to the degree of metal stress caused by the internal concentration of the toxic elements in plants (Cobbett 2000). PCs have an antioxidant property to reduce As toxicity in plants by chelation and its subsequent compartmentalization into vacuoles (Bleeker et al. 2003; Dago et al. 2014). Hartley-Whitaker et al. (2002) reported that PC plays a crucial role in detoxification of As in plants.

Upon entry, As affects multiple of biochemical and physiological attributes of which the most detrimental effect could be explained by modulation in photosynthetic efficacy (Gusman et al. 2013; Srivastava et al. 2013; Anjum et al. 2017). Previously, it has been reported that As stress resulted in growth inhibition with concomitant decrease in yield (Shahid et al. 2015; Chandrakar et al. 2016). Likewise, As interferes with chlorophyll synthesis and disrupts photosynthetic apparatus that consequently lowers effectiveness of PS-II (Li et al. 2007; Qadir et al. 2004; Bankaji et al. 2014). Additionally, As checks CO_2 entry by reduced stomatal conductance that is also associated with reduction in ability of gaseous exchange through transpiration (Milivojec et al. 2006; Anjum et al. 2016c). Under heavy metal stress alteration in carbon metabolism due to chlorophyll degradation and disturbances in electron transfer between PS-I and PS-II affecting nutritional value of agronomically important crops (Larbi et al. 2002; Wahid et al. 2007). Sugar molecules, the resultant product of carbon assimilation act as a regulator of stress responses and play principle role in gene expression under abiotic stresses (Rosa et al. 2009; Lemoine et al. 2013). Moreover, in plants tolerance mechanism primarily depends on sugar signaling pathways that has been known as prerequisite for early seedling growth under abiotic stress (Ho et al. 2001). Hu et al. (2012) reported decreased lipid peroxidation rate in salinity exposed wheat seedlings by exogenous addition of glucose. Thus, studies associated with differential accumulation of sugar molecules under As stress in agronomically important crops are an emerging research field that could develop relevant strategies to confer resistance towards ROS instigated toxicity by regulating carbon partitioning and metabolism.

The genomic integrity of plants is challenged by various environmental stresses. Intensive oxidative stress generation within the cell instigates DNA damage that alters its coding properties and ultimately affects cellular functionality (Polyn et al. 2015; Manova and Gruszka 2015). One major consequence of oxidative DNA damage is genomic instability (Cooke et al. 2003; Majumder et al. 2018). Plants have evolved diverse strategies to ensure genomic stability for maintenance of normal growth and metabolism that varies from species to species

(Yoshiyama et al. 2013). Most successfully applied technique to measure extent of DNA damage is Randomly amplified polymorphic DNA (RAPD) with reference to changes in DNA band profiles between treated and non treated samples (Atienzar et al. 1999; Dogan et al. 2016). Alteration in DNA band profiles scored as reduction in genomic template stability (GTS) (Taspinar et al. 2018). Decline in GTS values identifies the delicate changes in the genome and till now little is known about As(V) induced alteration in GTS value in terms of genotoxicity inspection in different rice cultivars. GTS values can be correlated with key fitness parameters to characterize tolerant and sensitive cultivars thereby, holds significant position in environmental safety. Thus, screening of tolerant and sensitive cultivars by physiological and molecular variables together is important for understanding the As tolerance mechanism of rice and can be the starting point for developing strategies for reducing arsenic uptake by most commonly consumed rice cultivars.

Rice is the most important cereal food of West Bengal and its cultivation requires water logging condition where AsIII is present as predominant form (Riveros and Figures 2000; Mohan and Pittman 2007). Several strategies have been initiated to improve the adaptability of rice plants to withstand As stress. Norton et al. (2009) screened 76 rice cultivars of Bangladesh on the basis of total arsenic accumulation and identified the cultivars with least arsenic content. Kuramata et al. (2011) reported genotypic differences with respect to total arsenic accumulation in grains of 10 japanese rice cultivars. Screening of four wheat cultivars for arsenic uptake, its accumulation and tolerance was conducted by both Kundu et al. (2012) and Noor et al. (2016) who reported significant variation due to differences in morphological attributes. Therefore, identification of arsenic tolerant and sensitive cultivars by using physiological, biochemical and molecular parameters together holds a significant position, required to minimize arsenic toxicity. This knowledge along with genetic researches can be used in breeding programmes to identify the genes responsible for decreasing trivalent arsenic in aerial parts and grains of tolerant cultivars and can be used to predict the mechanism associated with their tolerance/sensitivity.

The aim of the present study was to identify and characterize arsenic tolerant and sensitive rice genotypes grown in West Bengal on the basis of arsenic accumulation, rate of its conversion to arsenite and their influence on growth, water contents, accumulation of stress markers, antioxidants activities, ascorbate-glutathione cycle, thiol metabolism, chloroplastic pigments, gas exchange attributes, carbohydrate metabolism and its further validation by genomic profiling.

Objectives of the project

1) **To identify and characterize arsenic sensitive and tolerant rice genotypes grown in West Bengal on the basis of arsenic accumulation, its influence on growth and metabolism using biochemical parameters and its further validation by genomic profiling.**

2) **To study the role of glutathione and phytochelatins in combating arsenic toxicity in the test cultivars.**

Summary of the findings

- In the present study, arsenate exposure adversely affected the growth and water contents in the twelve rice cultivars. Effects were severe in cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20 than cvs. Bhutmuri, Kumargore, Binni, Vijaya, TN-1 and IR-64. Higher accumulation of total arsenic and arsenite in cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20 under arsenate challenged condition led to H_2O_2 generation coupled with enhanced level of lipid peroxidation that resulted in growth retardation.

- Arsenate toxicity reduced the activity of catalase whereas superoxide dismutase activity increased along with increase in the levels of oxidative stress markers viz. H_2O_2, proline and MDA. The differential alteration in the activities of antioxidant enzymes and levels of oxidative stress markers were more pronounced in cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20, suggesting these cultivars suffered more oxidative damage.

- Rice cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20 were characterized by accumulation of higher levels of GSH & ascorbate compared to cvs. Bhutmuri, Kumargore, Binni, Vijaya, TN-1 and IR-64. The higher activities of glutathione reductase, glutathione-S-transferase, glutathione peroxidase and ascorbate peroxidase were recorded in cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20 than cvs. Bhutmuri, Kumargore, Binni, Vijaya, TN-1 and IR-64. Higher activity of APX demonstrates that ascorbate– glutathione cycle plays an important role under arsenate exposure. In the study, it was demonstrated that in As(V) treated seedlings, significant amount of As(V) was converted to As(III) by increased activity of arsenate reductase. This reduction was coupled to NADPH oxidation via the reduction of

GSSG to GSH by escalated activity of glutathione reductase in cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20. Thus it can be concluded that these cultivars exhibiting sensitivity towards arsenic toxicity.

- Arsenate exposure led to an increase in ascorbate contents along with increased activity of APX enzyme in twelve test cultivars with more increments in cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20 compared to cvs. Bhutmuri, Kumargore, Binni, Vijaya, TN-1 and IR-64. This could be correlated with the presence of decreased levels of total arsenic, arsenite and H_2O_2 in the latter six cultivars, resulted less oxidative damage leading to better growth and development of the test seedlings in response to arsenate stress.

- In the study, a significant elevation in PCs production was observed in As(V) treated seedlings of cvs. Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20 that correlated with the accumulation of higher level of total As and an elevation in the activity of AR enzyme, caused rise in As(III) species which resulted in the production of escalated amounts of phytochelatins to reduce As(III) induced oxidative damage in the rice seedlings.

- Generation of higher oxidative stress under total As and As(III) accumulation in rice cultivars Swarnadhan, Tulaipanji, Pusa basmati, Badshabhog, Tulsibhog and IR-20 due to insufficient activity of enzymatic and non-enzymatic antioxidants resulted in depletion of chlorophyll contents along with significant reduction in carotenoid contents that eventually led to pronounced inhibition of Hill activity.

- In the study, gas exchange attributes viz., net photosynthetic rate (*Pn*), internal CO_2 concentration (*Ci*), stomatal conductance (*gs*) and transpiration rate (*E*) reduced with increase in As(V) concentration. Poor intercellular CO_2 level (*Ci*) under As exposure might be attributed to stomatal closure associated with pronounced reduction in Pn. being maximum in cv. Swarnadhan followed by cvs. Tulaipanji, Pusa basmati, Badshabhog, tulsibhog and IR-20 and characterized with lowered *Pn* associated reduction in growth and vigour than cvs. Bhutmuri, Binni, Kumargore, Vijaya, TN-1 and IR-64.

- In the study, data obtained from RAPD analysis demonstrates that significant changes occurred with the appearance and disappearance of RAPD band profiles of arsenic-untreated and treated seedlings and said variations markedly differed among twelve tested cultivars. The highest alterations in RAPD profiles along with reduction in GTS values were lower in cvs. Bhutmuri, Binni, Kumargore, and Vijaya while cvs. Swarnadhan, Pusa basmati, Badshabhog, and Tulsibhog exhibited higher level of reduction in GTS values and showed their sensitivity to arsenic toxicity.

- **Out of twelve cultivars tested, cvs. Bhutmuri and Kumargore exhibited the highest degree of tolerance while cvs. Swarnadhan and Tulaipanji showed highest degree of sensitivity to arsenic. Thus, in future, identified arsenic- tolerant/less sensitive cultivars could be used to understand the mechanism of tolerance along with that they could be used in breeding program to enhance arsenic tolerance by transferring trait to sensitive cultivars. In addition to that identified tolerant cultivars could be cultivated in arsenic prone areas of West Bengal to minimize arsenic toxicity.**

Contribution to the society

The data presented in the study will advance the knowledge in the field of ecotoxicology and As induced genotoxicity. The information acquired on As uptake, accumulation and its translocation will help to frame a strategy to develop As free rice plants. The low As accumulating rice cultivars especially, Bhutmuri and Kumargore could be used in conventional breeding programme as the source of germplasm to restrict As uptake and its translocation within plant body to develop As free rice cultivar for safer human consumption. Additionally, cultivation of low As accumulating cultivars with desired agricultural execution would be the cost effective way to minimize As contamination associated health hazards in As contaminated soil of West Bengal.

Publications from the project work

1. **B. Majumder**, S. Das, S. Mukhopadhyay and A. K. Biswas (2018) : Identification of arsenic tolerant and arsenic sensitive rice (*Oryza sativa* L.) cultivars on the basis of arsenic accumulation assisted stress perception, morpho-biochemical responses, and alteration in genomic template stability. ***Protoplasma*** – Springer (I.F. 2.46) 255: 1-19

2. S. Das, **B. Majumder**, A. K. Biswas (2018) : Modulation of growth, ascorbate-glutathione cycle and thiol metabolism in rice (*Oryza sativa* L. cv. MTU-1010) seedlings by arsenic and silicon. ***Ecotoxicology*** – *Springer* (I.F. 1.99) 27: 1387-1403

3. **B. Majumder** and A. K. Biswas (2018) : Polyamines: Role in attenuation of heavy metal toxicity. ***Kongonadu Research Journal*** 5(1): 60-63

4. J. Saha, **B. Majumder**, B. Mumtaz and A. K. Biswas (2017): Arsenic induced oxidative stress and thiol metabolism in two cultivars of rice (*Oryza sativa* L.) and its possible reversal by phosphate. ***Acta Physiologiae Plantarum*** – Springer (I.F. 1.44) 39 : 263

Annexure – X

UNIVERSITY OF GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110002

ASSESSMENT CERTIFICATE

It is certified that the proposal entitled **"Screening of arsenic tolerant and sensitive rice (*Oryza sativa* L.) cultivars ……………………on the basis of tolerance, growth and metabolism"** by Prof. Asok K. Biswas Department of Botany has been assessed by the Expert committee consisting the following members for submission to the University Grants Commission, New Delhi for financial support under the scheme of Major Research Project.

Details of Expert Committee:	**Comments**
Dr. Aloke Bhattacharjee Professor (Retd.) Plant Physiology and Biochemistry Department of Botany, University of Burdwan. Pin -713104 E-mail: alokebc@yahoo.co.in	I have gone through the details of the work done and the net outcome of the Project. After critical assessment I am under impression that it is an excellent piece of work having immense importance in agricultural aspect. The quality of the work done is also reflected from the valuable publications from the Project work. Aloke Bhattacharjee A. BHATTACHARJEE (Retd.) Prof. & Head Department of Botany BURDWAN UNIVERSITY BURDWAN-713104
Dr. Gaurab Gangopadhyay Professor Division of Plant Biology Main Campus, Bose Institute, Kolkata - 700 009, India E-mail:gaurab@jcbose.ac.in	I have gone through the work report of the project. It is an extremely well-executed research work on a very pertinent issue, viz. arsenic tolerance vis-a-vis susceptibility of rice in the backdrop of W. Bengal, a severely Arsenic prone area. The results so far obtained are exciting and leading to the next level of research. The workers have already published few papers in journals of repute and, there is scope for further publications. Gaurab Gangopadhyay 25.05.2019

The proposal is as per the guidelines.

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ORIGINAL ARTICLE

Identification of arsenic-tolerant and arsenic-sensitive rice (*Oryza sativa* L.) cultivars on the basis of arsenic accumulation assisted stress perception, morpho-biochemical responses, and alteration in genomic template stability

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Abstract

Arsenic toxicity is the most commonly experienced challenge of rice plants due to irrigation with arsenic-polluted groundwater and their cultivation in water logging environment which poses threat to human health, particularly in Bangladesh and West Bengal (India). In the present study, hydroponically grown eight rice cultivars, viz., Bhutmuri, Kumargore, Binni, Vijaya, Tulsibhog, Badshabhog, Pusa basmati, and Swarnadhan, were screened for arsenic tolerance by using physiological and molecular parameters. Treatment with 25 μM, 50 μM, and 75 μM arsenate resulted in dosage-based retardation in growth and water content in all the tested cultivars due to accumulation of total arsenic along with the enhanced activity of arsenate reductase with more severe effects exhibited in cvs. Swarnadhan, Pusa basmati, Badshabhog, and Tulsibhog. Arsenic sensitivity of rice cultivars was evaluated in terms of oxidative stress markers generation, antioxidant enzyme activities, and level of genotoxicity. Under arsenate-challenged conditions, the levels of oxidative stress markers, viz., H_2O_2, MDA, and proline, and activities of antioxidant enzymes, viz., SOD and CAT, along with the level of genotoxicity analyzed by RAPD profiling were altered in variable levels in all tested rice cultivars and showed a significant alteration in band patterns in arsenate-treated seedlings of cvs. Swarnadhan, Pusa basmati, Badshabhog, and Tulsibhog in terms of appearance of new bands and disappearance of normal bands that were presented in untreated seedlings led to reduction in genomic template stability due to their high susceptibility to arsenic toxicity. Cultivar- and dose-dependent alteration of parameters tested including the rate of As accumulation showed that cvs. Kumargore, Binni, and Vijaya, specially Bhutmuri, were characterized as arsenate tolerant and could be cultivated in arsenic-prone areas to minimize level of toxicity and potential health hazards.

Keywords Arsenate · Rice cultivar · Screening · Oxidative stress · RAPD · GTS

Abbreviations

ANOVA	One-way variance
As	Arsenic
AsIII	Arsenite
AsV	Arsenate
CAT	Catalase
DNA	Deoxyribonucleic acid
DW	Dry weight
FW	Fresh weight
EU	Enzyme unit
GTS	Genomic template stability
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
mM	Millimolar
μM	Micromole
RAPD	Random amplified polymorphic DNA
ROS	Reactive oxygen species
SOD	Superoxide dismutase

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Ecotoxicology
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Modulation of growth, ascorbate-glutathione cycle and thiol metabolism in rice (*Oryza sativa* L. cv. MTU-1010) seedlings by arsenic and silicon

Susmita Das[1] · Barsha Majumder[1] · Asok K. Biswas[1]

Accepted: 15 October 2018

Abstract
Arsenic is a carcinogenic metalloid, exists in two important oxidation states—arsenate (As–V) and arsenite (As-III). The influence of arsenate with or without silicate on the growth and thiol metabolism in rice (*Oryza sativa* L. cv. MTU-1010) seedlings were investigated. Arsenate was more toxic for root growth than shoot growth where the root lengths were short, characteristically fragile and root tips turned brown. The multiple comparison analysis using Tukey's HSD (honest significant difference) tests indicated that the rate of arsenate accumulation and its conversion to arsenite by arsenate reductase were significantly increased in all arsenate treated seedlings while in seedlings treated jointly with arsenate and silicate, arsenate accumulation and its conversion to arsenite decreased. Silicate content was detected in the seedlings treated with silicate alone and under co-application of arsenate with silicate. In the test seedlings arsenic toxicity increased ascorbate and glutathione contents along with the activities of their regulatory enzymes, viz., ascorbate peroxidase, glutathione reductase, glutathione peroxidase and glutathione–s-transferase to reduce the toxicity level induced by arsenic whereas ascorbate oxidase activity was decreased to maintain sufficient ascorbate pool under arsenate treatment. Phytochelatins production were increased in both root and shoot of the test seedlings under arsenate exposure to alter the detrimental effects of arsenic by chelation with arsenite and their subsequent sequestration into vacuole. Thus, joint application of silicate along with arsenate showed significant alterations on all the parameters tested compared to arsenate treatment alone due to less availability of arsenic in the tissue leading to better growth and metabolism in rice seedlings. Thus use of silicon in arsenic contaminated medium may help to grow rice with improved vigour.

Keywords Arsenic · Rice · Silicon · Arsenate reductase · Glutathione · Phytochelatin

Abbreviation

AA	ascorbic acid or ascorbate
AOX	ascorbate oxidase
ACN	acetonitrile
APX	ascorbate peroxidase
AR	arsenate reductase
As	arsenic
As(V)	arsenate
As(III)	arsenite
BSA	bovine serum albumin
$Ca(NO_3)_2$	calcium nitrate
CDNB	1-chloro-2,4-dinitrobenzene
$CuSO_4$	copper sulfate
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
DNPH	2,4-dinitrophenylhydrazine
DTNB	5,5-dithiobis-2-nitrobenzoic acid
DTPA	diethylene triamine penta acetic acid
DTT	dithiothreitol
DW	dry weight
EDTA	ethylene diamine tetra acetic acid
FW	fresh weight
GSH	glutathione reduced
GSSG	glutathione oxidized
GPx	glutathione peroxidase
GR	glutathione reductase

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10646-018-1994-5) contains supplementary material, which is available to authorized users.

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ORIGINAL ARTICLE

Arsenic-induced oxidative stress and thiol metabolism in two cultivars of rice and its possible reversal by phosphate

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Abstract Groundwater arsenic contamination, a grave threat in Bangladesh and parts of West Bengal (India), causes biochemical and physiological disorders in plants. Arsenic and phosphorus (plant macronutrient) have similar electronic configurations, resulting in their competitive interaction for the same uptake system in plant roots. Arsenic exposure initiates production of reactive oxygen species. Hence, the contents of proline, hydrogen peroxide, glutathione, ascorbate, and activities of ascorbate peroxidase, catalase were investigated in 21-day-old rice seedlings (cv. Khitish and cv. Nayanmani). Additionally, impact of arsenate together with phosphate on growth, total glutathione contents and activity of its regulatory enzymes were altered in the test cultivars to varying extents. Inductively coupled plasma-optical emission spectroscopic study of arsenic content in the root and shoot also showed variable uptake of arsenic by the two cultivars. Arsenate reductase enzyme activity primarily observed in the root, also differed from one cultivar to the other. Different phytochelatin (PCs) levels were recorded in the shoot and root of the cultivars under arsenate and phosphate treatment by reverse phase-high performance liquid chromatography. PC content increased with increasing arsenate concentrations, whereas phosphate and arsenate co-application resulted in reduced PC levels. The degree of elevation in PC contents varied significantly in the cultivars. Based on the above-mentioned parameters, cv. Khitish appeared to be more susceptible to arsenic toxicity than cv. Nayanmani which showed selective tolerance to the said metal stress.

Keywords Arsenic · Glutathione · Oxidative stress · Phytochelatin · Rice

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Introduction

Heavy metal pollution due to arsenic (As) contamination affects millions especially in the southern regions of Asia where groundwater polluted with the metalloid is used for human consumption (Chakraborti et al. 2002; Nordstrom 2002). In India (particularly West Bengal) and neighboring Bangladesh chronic As poisoning has reached a massive scale (Bhattacharjee 2007) since As-enriched groundwater is abundantly utilized to cultivate rice fields (Ma et al. 2008). In humans, exposure to inorganic arsenic can harm the circulatory and nervous systems, lead to hyperkeratosis, pigmentation and in extreme cases several kinds of cancer (Sharma 2013). Research shows that rice is among the leading sources of inorganic As contamination to populations that consume rice daily (Kile et al. 2007; Ohno et al. 2007; Mondal and Polya 2008). Inflated amounts of As has been found in rice as this crop accumulates more As than any other dietary crop. This has become an impending threat for the Southeast Asian population. Arsenic primarily accumulates in roots and to a lesser degree in the aerial portion. This hinders the growth, reduces biomass (Stoeva et al. 2003), damages the physiological processes (Wells and Gilmor 1997) and decreases crop productivity (Stepanok 1998).

Roots are primarily responsible for arsenic uptake into the plants following which it is promptly converted to arsenite in the cytosol by arsenate reductase (AR) (Meharg

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