Plant Physiology and Biochemistry 80 (2014) 203–210

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Arsenic stress in rice: Redox consequences and regulation by iron

Shwetosmita Nath [a], Piyalee Panda [a], Sagarika Mishra [b], Mohitosh Dey [b], Shuvasish Choudhury [c,*], Lingaraj Sahoo [b], Sanjib Kumar Panda [a]

[a] Plant Molecular Biotechnology Laboratory, Department of Life Science and Bioinformatics, Assam University, Silchar 788 011, India
[b] Department of Biotechnology, Indian Institute of Technology, Guwahati 781 039, Assam, India
[c] Central Instrumentation Laboratory, Assam University, Silchar 788 011, India

ARTICLE INFO

Article history:
Received 30 October 2013
Accepted 14 April 2014
Available online 21 April 2014

Keywords:
Antioxidants
Arsenic
Gene expression
Iron
Oxidative stress
Oryza sativa

ABSTRACT

Arsenic (As) contamination is a serious hazard to human health and agriculture. It has emerged as an important threat for rice cultivation mainly in South Asian countries. In this study, we investigated the effect of iron (Fe) supplementation on arsenic (As^V) induced oxidative stress responses in rice (*Oryza sativa* L.). Rice seedlings treated with As^V for 24 and 48 h in presence or absence of 2.5 mM Fe after which the root and shoot tissues were harvested for analysis. The results indicate significant ($p \leq 0.05$) reduction in root and shoot length/dry biomass. Supplementation of Fe showed improved growth responses under stress as compared to As^V alone. The scanning electron microscopy (SEM) analysis of roots under As^V treatment for 48 h showed major alterations in root structure and integrity, although no noticeable changes were observed in Fe − supplemented seedlings. Significantly high ($p \leq 0.05$) accumulation of As^V was observed in root and shoot after 24 and 48 h of stress. However, under Fe − supplementation As accumulation in root and shoot were considerably low after 24 and 48 h of As^V treatment. The hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content in both root and shoot increased significantly ($p \leq 0.05$) after 24 and 48 h of As^V treatment. In Fe − supplemented seedlings, the levels of H_2O_2 and MDA were considerably low as compared to As^V alone. Ascorbate (AsA) and glutathione (GSH) levels also increased significantly ($p \leq 0.05$) under As^V stress as compared to control and Fe-supplemented seedlings. Activities of catalase (CAT) and superoxide dismutase (SOD) were significantly ($p \leq 0.05$) high after 24 and 48 h of As^V treatment as compared to Fe-supplemented seedlings. The gene expression analysis revealed up-regulation of metallothionein (*MT1*, *MT2*) and nodulin 26-like intrinsic protein (*NIP2;1*) genes after 5d of As treatment, while their expressions were repressed under Fe-supplementation. Our results indicate that Fe regulates oxidative stress and promotes growth under As stress.

1. Introduction

Arsenic (As) is toxic metalloid which is widely distributed in aquatic and terrestrial ecosystem (Phillips, 1990). It is considered as a class I carcinogen and food chain contaminant (Zhao et al., 2010). It exhibits four different valencies (-III, 0, III and V) with several chemical forms. In soils, As is usually present as pentavalent arsenate (As^V) and trivalent arsenite (As^{III}), later being more toxic than its pentavalent form (Zhao et al., 2010). Both of these forms are inter-convertible depending upon the redox sate of the soil (Tripathi et al., 2007). Ground water contamination with As is a serious issue in many South East Asian countries including India, Bangladesh, China and Vietman. Rice being the major staple crop grown in these areas is affected by As toxicity, mainly due to irrigation with As-contaminated water (Zhao et al., 2010; Williams et al., 2007; Brammer and Ravenscroft, 2009). Rice is highly efficient in accumulating As in comparison to other crops like wheat and barley (Williams et al., 2007). High As accumulation capacity of rice poses immense health hazards to almost 50% of world population who are dependent on rice as their staple food (Norton et al., 2009; Tripathi et al., 2012). Studies have also shown that rice and rice-related products are also major source of As exposure in humans who are not directly exposed to As contaminated drinking water (Mondol and Polya, 2008; Rahman et al., 2009). Being a redox active metalloid, it stimulates the production of reactive oxygen species (ROS) by the inter-converting one form to other, causing oxidative stress (Tripathi et al., 2012; Hartley-Whitaker et al., 2001;

* Corresponding author. Tel.: +91 94351 75587.
E-mail addresses: shuvasish@gmail.com, shuvasish.choudhury@aus.ac.in (S. Choudhury).

http://dx.doi.org/10.1016/j.plaphy.2014.04.013
0981-9428/

Choudhury and Panda, 2004; Mylona et al., 1998; Shaibur et al., 2006; Singh et al., 2007; Shri et al., 2009). As interacts with sulfhydryl (-SH) groups of enzymes and proteins, leading to inhibition of several important cellular functions (Mehrag and Hartley-Whitaker, 2002). Studies have also shown that As (As^V) acts as phosphate analog and hinder ATP production and oxidative phophorylation (Tripathi et al., 2007).

Evidences from physiological studies suggest that As transport occurs via aquaporins in rice (Meharg and Jardine, 2003). Though, the exact mechanism of As transport in plants remain unclear, studies have revealed the possible role of nodulin26-like intrinsic proteins (NIPs) (Ali et al., 2009). In *Arabidopsis*, nodulin 26-like intrinsic proteins (NIPs) like NIP5; 1, 6; 1 and 7; 1 are involved in As transport and loss of NIP 7; 1 function leads to As tolerance (Isayenkov and Maathuis, 2008). Plants have also evolved several mechanisms to combat the increasing stress load. Such intrinsic mechanisms for counteracting As toxicity in plants include phytochelatin (PC) dependant detoxification vis-à-vis induction of sulfate uptake and reduction pathways (Rausch and Wachter, 2005). Higher level of PCs and PC-synthase activity along with coordinated thiol metabolism were reported in rice, which induce As tolerance (Tripathi et al., 2012). Further, PC-arsenite complexion in rice leaves reduces translocation of As from leaves to grains (Duan et al., 2011). In addition, the potential role of various metallothioniens (MTs) in arsenic detoxification in rice was also reported (Gautam et al., 2012).

Iron (Fe) is one of the major elements required by plants for normal growth and metabolism. It is an essential element for photosynthesis, respiration, DNA synthesis and co-factors for several enzymes (Hell and Stephan, 2003; Jeong and Connolly, 2009). In soil, it is mainly present in insoluble oxidized (Fe^{III}) form. In flooded rice a field, Fe is converted from Fe^{III} to ferrous (Fe^{II}) form and quickly released from the soil and sequester As (Takahashi, 2004). Studies have also shown that Fe plaque formation over root surface reduces As accumulation (Meharg, 2004). The role of Fe in controlling As toxicity in plants were reported in several plant species, most of which concerns As accumulation and speciation patterns (Meharg, 2004; Juskelis et al., 2013; Stone, 2008). The role of Fe in mediating As induced oxidative stress as a strategy for As tolerance have been poorly reported. In the present study, we investigate the role of Fe supplementation on growth and oxidative stress responses under As stress. To elucidate the ameliorative role of Fe, we evaluate growth responses, As-accumulation, ROS production, lipid peroxidation, antioxidant level and expression pattern of some important genes in rice.

2. Methods

2.1. Plant material and treatments

Viable rice seeds (cv: Ranjit) were procured from Regional Agricultural Research Station, Karimganj, India, surfaced sterilized with 0.1% mercuric chloride ($HgCl_2$) and washed thoroughly with deionised water. Sterile seeds were germinated over moistened filter papers in dark for 48 h. Uniformly germinated seeds were transferred to plastic cups containing 350 ml Hoagland nutrient solution at pH 6.2 (Hoagland and Arnon, 1950) and grown for 5d under white light (52 $\mu Em^{-2}\ s^{-1}$ PAR) at 25 °C with 16 h photoperiod. Lethal dose (LD50) was measured for determining the concentration of As [sodium arsenate (Na_2AsO_4)] and Fe [ferric chloride ($FeCl_3$)] (see Supplementary Fig. S1–S2). Based on LD50 results ($LD50_{As} = 200\ \mu M$; $LD50_{Fe} = 3.5$ mM) final concentration of As (0 and 100 μM) and Fe (2.5 mM) were selected. Two different sets, either in presence or absence of Fe were prepared and As (100 μM) was added to the nutrient solution. After 24 and 48 h, root and shoot were used for analysis.

2.2. Growth, uptake and SEM analysis

The root and shoot length were measured for five plants per treatment after 24 and 48 h using a centimeter scale. To measure the dry biomass, 1 g of plant material was dried at 80 °C for 48 h and weighed. For measurement of As uptake, tissue samples were briefly rinsed with deionised water and the excess water was soaked out with a paper towel. Sample was dried as mentioned above. 100 mg of dried sample was digested with 5 ml acidic mixture of nitric acid (HNO_3) and hydrochloric acid (HCl) in the ratio of 3:1. After complete digestion, the final volume was adjusted to 20 ml with deionised water. The total As and Fe content was recorded using Atomic Absorption Spectrometer (3110/AAnalyst 200, Perkin Elmer, USA). For scanning electron microscopy (SEM) analysis, root samples were fixed in 2.5% glutaraldehyde in 0.2 M sodium phosphate buffer for 2 h at 4 °C. The post fixation, staining and SEM analysis were carried out at Electron Microscopy Division, Sophisticated Analytical Instrumentation Facility at NEHU, Shillong.

2.3. Hydrogen peroxide and lipid peroxidation

Hydrogen peroxide (H_2O_2) content was determined as per the method of Sagisaka (1976). Briefly, 200 mg of tissue sample was homogenized with 10% (w/v) trichloroacetic acid (TCA) and centrifuged at 17, 000 g for 15min at 4 °C. The assay mixture contained 1.6 ml supernatant tissue extract, 0.4 ml 50% (w/v) TCA, 0.4 ml ferrous ammonium sulfate ($FeNHSO_4$) and 2.5 mM potassium thiocyanate (KSCN). The absorbance was recorded at 480 nm.

Lipid peroxidation was measured as per the method of ZhangZhang (1992) in terms of malondialdehyde (MDA) content. 200 mg of tissue sample was homogenized in 0.25% (w/v) thiobarbituric acid (TBA) prepared in 10% (w/v) TCA. The extract was heated at 95 °C for 30min and cooled in ice. The mixture was centrifuged at 10, 000 g for 10min at 4 °C and absorbance of the supernatant was recorded at 532 nm. The non-specific turbidity was corrected by subtracting the absorbance value at 600 nm.

2.4. Catalase (CAT) and superoxide dismutase (SOD) activities

The enzymes from plant tissues were extracted with sodium phosphate buffer (0.1 M, pH 6.8) and centrifuged at 17, 000 g at 4 °C for 15min. The supernatant obtained was used for assay of enzymes. The reaction mixture of CAT (EC 1.11.1.6) contained 2 ml sodium phosphate buffer (0.1 M), 0.5 ml H_2O_2 (30 mM) and 0.5 ml sterile distilled water. The reaction mixture was incubated for 1min and the absorbance was recorded at 240 nm. The CAT activity was expressed as U $min^{-1}\ g^{-1}$ f.w. using an extinction coefficient of 43.6 $mM^{-1}\ cm^{-1}$ (Chance and Maehly, 1955). The reaction mixture for SOD (EC 1.15.1.1) contained tris–HCl buffer (79.2 mM, pH 6.8), EDTA (0.12 mM), tetraethylenediamine (10.8 mM), bovine serum albumin (0.0033%) nitroblue tetrazolium (600 μM in 5 mM KOH) and 0.2 ml supernatant enzyme extract. The reaction was initiated by placing the glass vials under fluorescent light (20 W Phillips, India). By switching on the light on and off, the reaction was initiated and terminated. The absorbance for formazan formation was recorded at 560 nM. The activity was expressed as $\Delta A_{560}\ g^{-1}$ (fr.wt.)10 min^{-1} (Giannopolitis and Ries, 1980).

2.5. Ascorbate (AsA) and glutathione (GSH) content

For the estimation of ascorbate (AsA) and glutathione (GSH), 200 mg of the plant tissue was homogenized in sterile distilled water and centrifuged at 17,000 g for 15min at 4 °C. For the estimation of AsA content, the reaction mixture contained sodium molybdate (2%w/v), sulfuric acid (0.1N H_2SO_4), sodium phosphate