Ecotoxicology and Environmental Safety 107 (2014) 46–54

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Arsenate induced differential response in rice genotypes

Meetu Gupta*, Mohd. Anwar Ahmad

Ecotoxicogenomics Lab, Department of Biotechnology, Jamia Millia Islamia, New Delhi-110025, India

ARTICLE INFO

Article history:
Received 30 October 2013
Received in revised form
17 April 2014
Accepted 21 April 2014
Available online 4 June 2014

Keywords:
Antioxidant enzymes
Arsenic
Gene expression
Oryza sativa
Protein profile

ABSTRACT

To study the differential response in two rice genotypes (PB1 and IR-64), hydroponically grown 14 days old plants were exposed to 50, 150 and 300 μM As(V) for 24 and 96 h. Accumulation of As was not significantly higher in PB1 variety except at higher concentration (300 μM) and duration (96 h), but up regulation of gene transcripts were higher as compared to IR-64. Inhibition in seed germination, root–shoot length, chlorophyll and protein content was observed in both varieties with increasing concentration and exposure time. PB1 variety was found more capable to detoxify As(V) through induction of antioxidant defense system and other stress related parameters (cysteine, proline content). SDS-PAGE and semi quantitative RT-PCR analysis showed significant changes in protein profile and gene expression analysis. The results suggests that various studied parameters and transcripts accumulation showed a combinatorial type of tolerance mechanism in PB1 variety to provide better protection against As (V) stress.

1. Introduction

Plants encounter a wide range of environmental stresses (abiotic and biotic) during their life cycle. Among abiotic stresses, the hazards caused by heavy metals and metalloids are one of the major concern. Heavy metals, depending on their oxidation states, can be highly reactive and as a consequence, toxic to most organisms. Among all the metal/metalloid pollutants, arsenic (As) is one of the most hazardous elements of real concern to human health. It is a metalloid and occurs ubiquitously in nature. It is found predominantly as inorganic arsenate (As(V)) and arsenite (As(III)) forms. Arsenate, as a phosphate analogue, has detrimental effects on phosphate metabolism and arsenite is toxic due to its high reactivity with sulfhydryl groups (Zhu et al., 2008). Both forms of As are inter convertible depending on the redox condition of the soil. Arsenic is classified as a group 1 carcinogen and there is large concern about human contact with As that occurs through contaminated water and via the food chain.

Plants being sessile have developed unique molecular mechanisms to cope with different stress factors. However, variations do exist in tolerance mechanisms in plants. The only option for plants is to alter their physiologies, metabolic mechanisms, gene expressions and developmental activities to cope with the stress effects. Previous studies revealed novel insights into the plant defense mechanisms and the regulation of genes and gene networks in response to As toxicity (Tripathi et al., 2012a). The differential expression of transcripts encoding glutathione-*S*-transferases, antioxidants, sulfur metabolism, heat-shock proteins, metal transporters, and enzymes in the ubiquitination pathway of protein degradation as well as several unknown novel proteins serve as molecular evidence for the physiological responses to As stress in plants (Paulose et al., 2010). Arsenic exposed plants shows interruption in several physiological, biochemical and molecular processes, which includes inhibition in germination, reduction in root–shoot length, generation of reactive oxygen species and lipid peroxidation (Ahmad et al., 2012; Tripathi et al., 2012b). Arsenate (As(V)), the dominant form of As in aerobic condition, is taken up by the plants through phosphate transporters and interferes with glycolysis oxidative phosphorylation, causes cellular damage through ROS signaling pathways and induces alteration in gene expression (Rai et al. 2011).

Rice is the main dietary crop of about three billion people of the world and 90 percent of world's rice is produced and consumed in Asian countries (Stone, 2008). India is the world's second and Bangladesh is the fourth largest rice producer, due to this reason most of the rice of the world consumed in these two countries contributes to about half of the total As intake (Ohno et al., 2007; Mondal and Polya, 2008; Meharg et al., 2009). Rice grains accumulate more As compared to other cereals crops because of its method of cultivation in anaerobic soil where As is readily available. (Abedin et al., 2002).

Since As shares the transport system with essential and beneficial elements, it is not feasible to block completely its entry into crop plants. One approach of reducing the adverse effect is to

Abbreviations: As, arsenic; As(V), arsenate; APX, ascorbate peroxidase; CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde; PB 1, Pusa Basmati 1; RT-PCR, reverse transcriptase polymerase chain reaction

* Corresponding author.
E-mail address: meetu_gpt@yahoo.com (M. Gupta).

http://dx.doi.org/10.1016/j.ecoenv.2014.04.030
0147-6513/

select crop plants tolerant to As species. In the present work, the level of tolerance and detoxification strategy was studied through the analysis of antioxidant enzymes, stress related parameters and gene expression patterns in two rice (*Oryza sativa*) varieties namely, IR-64 and PB1. These two rice varieties are very commonly grown and consumed in Indian subcontinent.

2. Experimental

2.1. Plant growth and stress treatment

Seeds of two varieties of rice (PB1 and IR-64) were obtained from Indian Agriculture Research Institute, Pusa, New Delhi, India. Seeds were surface sterilized in 3 percent H_2O_2 and washed with distilled water prior to germination on a moist cotton bed and watered with 10 percent Hoagland nutrient medium with and without As(V) (prepared using salt Na_2HAsO_4) and kept in dark for 2 days. The seedlings were transferred to light (16 h photoperiod) with a day/night temperature of 25 ± 2 °C. Fourteen days old plants were treated with different concentrations of As(V) (50, 150 and 300 μM) for 24 and 96 h duration and solution was changed after every two days. After harvesting each plant was separated into leaf and roots, washed thoroughly with distilled water, frozen in liquid nitrogen and kept in −80 °C for biochemical and molecular analysis. Plants treated without metals served as controls. Each experiment was carried out in triplicates and each replicate contained equal number of seedlings (10–15) of same age. Percentage of seed germination and reduction in shoot/root length were calculated using the formula: percent seed germination = germinated seed/total number of seeds × 100 and percent reduction in shoot/root length = control-treated/control × 100, respectively.

2.2. Arsenic accumulation

Oven dried equal amount of leaves and root samples were powdered and digested in 2 ml of concentrated HNO_3. The digestion tubes were heated at 120 °C for 5 h. For quality assurance and quality control, the appropriate number of blank and standard reference material sample (Rice Flour: Item number 1568A, from NIST, USA) was included in the digestion procedure and further analysis. After digestion residue was taken up in the 10 ml of 10 percent (w/v) HCl, containing 10 percent (w/v) KI and 5 percent (w/v) ascorbic acid. Total As samples were then determined by hydride generation atomic absorption spectrophotometry (AA 6800, Shimadzu) coupled to a GBC hydride generation system using external calibration through arsenate.

Translocation factor (TF) was calculated using the following formula:

$$TF = \frac{[As]_{shoot}}{[As]_{root}},$$

$[As]_{shoot}$: As concentration of shoot (mg kg^{-1}), $[As]_{root}$: As concentration of root (mg kg^{-1})

2.3. Chlorophyll and protein estimation

Total chlorophyll content was estimated following the method of Arnon (1949). Treated and control leaves (100 mg) were homogenized in 80 percent chilled acetone, centrifuged at 10,000 rpm at 4 °C for 10 min and supernatant was used for chlorophyll estimation. Absorbance was read at 663 and 645 nm using Optizen 3220 UV^{bio} spectrophotometer (Mecasys, Korea). Protein estimation was carried out following Bradford (1976) using BSA as a standard. The absorbance of the sample was measured at 595 nm.

2.4. Enzymatic cellular antioxidants

Fresh leaf tissues (0.2–0.5 mg) of treated and control plants, frozen in liquid nitrogen and homogenized in ice cold 2 ml of 0.5 M potassium phosphate buffer (pH 7.3) containing 1.0 g PVP, 1.0 ml Triton-X100 and 0.11 g EDTA in pre-cooled mortar and pestle, centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was used for the measurement of enzyme activity (SOD and CAT).

2.4.1. Superoxide dismutase (SOD)

SOD activity was assayed according to the method of Beauchamp and Fridovich (1971). Assay mixture of 2 ml contained 100 μl enzyme extract, 1 M Na_2CO_3 (pH 10.2), 10 mM riboflavin, 2.0 mM L-methionine and 1.72 mM nitroblue tetrazolium chloride (NBT in 50 mM sodium-PBS, pH 7.8). The difference in increase was measured at 560 nm in the presence and absence of extract. Enzyme extract was proportional to the amount of enzyme. SOD activity was expressed as unit EU min^{-1} mg^{-1} protein. One unit of SOD was defined as the amount of enzyme required to cause 50 percent inhibition in the rate of NBT photo-reduction.

2.4.2. Catalase (CAT)

Catalase activity was assayed according to the method of Aebi (1983). Assay mixture in a total volume of 1 ml contained 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 60 mM H_2O_2 and 100 μl enzyme extract. The rate of H_2O_2 decomposition was measured at 240 nm (extinction coefficient of 0.036 mM^{-1} cm^{-1}) and enzyme specific activity was expressed as μmoles H_2O_2 oxidized min^{-1} mg^{-1} protein.

2.4.3. Ascorbate peroxidase (APX)

Ascorbate peroxidase activity was measured according to the method of Nakano and Asada (1981). Leaf samples (0.2–0.5) homogenized in medium containing 100 mM phosphate buffer (pH 7.3), 1 mM EDTA, 1 percent PVP and 1 mM ascorbate. The rate of H_2O_2 dependent oxidation of ascorbic acid (AA) was determined in a reaction mixture contained 0.1 M phosphate buffer (pH 7.0), 0.5 mM AA and 100 μl enzyme extract. Oxidation rate of AA was estimated by following the decrease in the absorbance at 290 nm for every 3 min. APX activity was calculated by using the extinction coefficient 2.8 mM^{-1} cm^{-1} and expressed in EU min^{-1} mg^{-1} protein.

2.5. Stress related parameters

2.5.1. Cysteine, MDA and proline estimation

The method of Gaitonde (1967) was followed for the estimation of cysteine. Leaves (500 mg) were crushed in 5 ml of chilled perchloric acid ($HClO_4$) and centrifuged at 10,000 rpm for 20 min. Cysteine content was measured in supernatant using acid ninhydrin reagent at 560 nm.

Malondialdehyde (MDA) content was estimated following Heath and Packer (1968) by reaction with thiobarbituric acid (TBA). The amount of MDA was calculated from the difference in absorbance at 532 and 600 nm using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Level of proline was measured following Bates et al. (1973). Plant leaves (0.5 g) were crushed in 3 percent sulfosalicylic acid and centrifuged at 4000 × *g* for 10 min. To 2 ml of supernatant, 2 ml of ninhydrin was added with 2 ml acetic acid and incubated at boiling temperature for 1 h. The mixture was extracted with toluene, and proline was quantified spectrophotometrically at 520 nm from the organic phase.