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Comparative transcriptome analysis of arsenate and arsenite stresses in rice seedlings

Debasis Chakrabarty [1], Prabodh Kumar Trivedi [1], Prashant Misra, Manish Tiwari, Manju Shri, Devesh Shukla, Smita Kumar, Arti Rai, Ashutosh Pandey, Deepti Nigam, Rudra Dev Tripathi, Rakesh Tuli *

National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, UP, India

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ABSTRACT

The effect of arsenic (As) exposure on genome-wide expression was examined in rice (*Oryza sativa* L., ssp. *Indica*). A group of defense and stress-responsive genes, transporters, heat-shock proteins, metallothioneins, sulfate-metabolizing proteins, and regulatory genes showed differential expression in rice seedlings challenged with arsenate (AsV) and arsenite (AsIII). AsV stress led to upregulation or downregulation of an additional set of genes in comparison to AsIII. Differential expression of several genes that showed the highest contrast in a microarray analysis was validated by following the quantitative changes in the levels of individual transcripts following challenge with AsV, AsIII, Cd, Cr, and Pb. Most of the selected genes responded to challenge by heavy metals such as arsenic. However, expression of one of the cytochrome P450 genes (Os01g43740) in rice root was induced by AsV but not by other heavy metals. Similarly, one glutaredoxin (Os01g26912) is expressed specifically in the AsIII-treated shoot.

1. Introduction

The presence of arsenic (As) in groundwater and food poses a serious health risk to people worldwide. The inorganic forms of the element, arsenite (AsIII) and arsenate (AsV), are more abundant in soils compared to the organic As species such as monomethylarsonic acid and dimethylarsonic acid (Tripathi et al., 2007). The mechanism by which arsenic is taken up by plants differs for AsIII and AsV. AsV is taken up by the high-affinity phosphate uptake system (Ullrich-Eberius et al., 1989), whereas AsIII uptake is thought to be accomplished through the aquaporins of the roots (Meharg and Jardine, 2003). Both inorganic forms of arsenic are highly toxic because they interfere with various metabolic pathways in cells such as the interaction of substrates/enzymes with the sulfhydryl groups of proteins and the replacement of phosphate in ATP for energy (Tripathi et al., 2007). Hence, plants exposed to arsenic show symptoms including decreases in both plant growth and crop yield (Carbonell Barrachina et al., 1995). In addition, arsenic stimulates the formation of free radicals and reactive oxygen species, resulting in oxidative stress (Requejo and Tena, 2005). Plants respond to AsV and AsIII stresses differentially by stimulating the antioxidant system and thiol metabolism, respectively (Mishra et al., 2008). In spite of many previous studies having been conducted on the effects of arsenic stress, the precise molecular mechanisms related to both the effects of arsenic phytotoxicity and the defense reactions of plants against arsenic exposure remain poorly understood at present. Recently, Dasgupta et al. (2004) have reported the association of As tolerance with the chromosome 6 in rice through quantitative trait loci (QTL) analysis. The study has been conducted using a doubled haploid population established by the anther culture of F_1 plants obtained from a cross between a Japonica cultivar, CJ06, and an Indica cultivar, TN1. Four QTLs present in rice chromosomes have been thus reported to be responsible for the uptake of As in different tissues and at various developmental stages (Zhang et al., 2008).

To characterize the arsenic-responsive genes, an analysis of the genome-wide expression of the rice transcriptome was carried out. A DNA microarray chip was used to identify the differentially expressed genes in rice following exposure to AsIII and AsV during germination and growth of the seedling. New genes identified in this study may provide more clues to understanding the molecular mechanism of response to As-induced stress in rice.

2. Materials and methods

2.1. Plant material, growth conditions, and treatments

Mature seeds of the Indica cultivar, IR64 (*Oryza sativa* L.) were manually dehusked, sterilized, and cultured on 1/2 Murashige and Skoog (MS) medium, containing either 25 μM AsIII ($NaAsO_2$,

* Corresponding author. Tel.: +91 522 2205841; fax: +91 522 205836.
E-mail address: rakeshtuli@hotmail.com (R. Tuli).
[1] Contributed equally to the results of this study.

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ICN, USA) or 250 μM AsV (Na_2HAsO_4, J.T. Baker, UK). Twenty seeds were placed in each plate, covered by a lid, and incubated under a 16-h light (110—130 μE m^{-2} s^{-1})/8-h dark photoperiod at 26 °C. For treatment with Pb [$Pb(NO_3)_2$; HiMedia, India), CrVI ($K_2Cr_2O_7$; SRL, India), and Cd ($CdCl_2$; Rankem, India), 100 μM of each element was used independently on the germinating seeds, as in the cases of AsV and AsIII. Shoot length, root length, root weight, and shoot weight were measured 10 days after sowing.

2.2. Total RNA extraction and transcriptome analyses

Total RNA from 10-d-old seedlings, grown with or without AsV or AsIII, was extracted using the QIAGEN RNeasy Plant Maxi Kit (QIAGEN, MD) for microarray analysis. Affymetrix GeneChip® Rice Genome Arrays (Gene Expression Omnibus platform Accession No. GPL2025) were used for microarray analyses. Target preparation, hybridization to arrays, washing, staining, and scanning were carried out according to the instructions of the manufacturer (Affymetrix, USA). Three independent replicated experiments were carried out for all the treatments. The hybridization data were analyzed using the Affymetrix GeneChip Operating Software (GCOS version 1.3). Satisfactory image files were analyzed to generate the probe intensity (.cel) files using the default settings of GCOS. Normalization of all the arrays was carried out using three independent methods: namely, probe logarithmic intensity error (PLIER), robust multiarray average (RMA), and GeneChip robust multiarray (GCRMA), using the ArrayAssist software (Stratagene, La Jolla, CA, USA). To identify statistically significant differentially expressed genes, a combined criterion of 2-fold or greater change and a P value <0.05 in the t-tests was adopted. Only common probe sets that were present in all the three analyses were considered significant. To obtain annotations for the arsenic-regulated probe sets, target sequences from the sequence information file of the rice genome array were searched using BLASTn against the TIGR rice pseudomolecules, release 5 (http://www.tigr.org/tdb/e2k1/osa1).

2.3. Pathway analysis

All the genes identified in the microarray analysis using the combined criterion of 2-fold or greater change and a P value <0.05 in the t-tests were used to generate the cellular-function pathways and interactomes using the software Pathway Studio (Ariadne Genomics, MD, USA). Pathways were generated for the genes modulated by AsV and AsIII independently. Another common pathway was generated for genes whose expression was modulated by both AsV and AsIII treatments.

2.4. Expression analysis using semiquantitative RTPCR

Rice plants were grown on ½MS medium containing AsV (250 μM), AsIII (25 μM), or other metals (Pb, Cr, and Cd; 100 μM), as described above. Total RNA from the root and the shoot of 10-d-old seedlings was extracted using the QIAGEN RNeasy Plant Maxi Kit (QIAGEN, MD), followed by treatment with RNAase-free DNase (Fermantas, Life Sciences, Ontario, Canada). RTPCR was carried out using 20 μL of the cDNA corresponding to the set of selected genes in a reaction containing 2X PCR Master mix (Fermantas, Life Sciences, Ontario, Canada). The list of selected genes and oligonucleotide primers (MWG, India) used in the study is provided as Supplementary Table S1. The primers for rice actin gene were used as loading control to ensure that equal amounts of cDNA were used in all the reactions. The PCR reaction was carried out using the following cycle conditions: an initial denaturation at 94 °C for 2 min, 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final 5-min extension at 72 °C.

3. Results and discussion

3.1. Growth of rice seedlings exposed to arsenic

Seeds germinated on media containing different concentrations of AsV and AsIII show a decline in germination and retardation in the growth of the seedlings during the 10 days of observation. The toxic effect was much higher in AsIII in comparison to AsV (data not shown). In our study, we observed similar toxic effects in AsV (250 μM) or ASIII (25 μM) with a significant decline (40–50%) in the length and weight of roots (Fig. 1A and B) demonstrating iso-toxic effect. Our study as well as results from other groups clearly suggest that AsIII is more toxic in comparison to AsV (Fitz and Wenzel 2002; Abedin and Meharg, 2002; Meharg and Hartley-Whitaker 2002; Dembitsky and Rezanka 2003; Liu et al., 2004; Mahimairaja et al., 2005). Reduced plant growth in response to arsenic exposure has also been reported by numerous investigators in other plants (Liu et al., 2005).

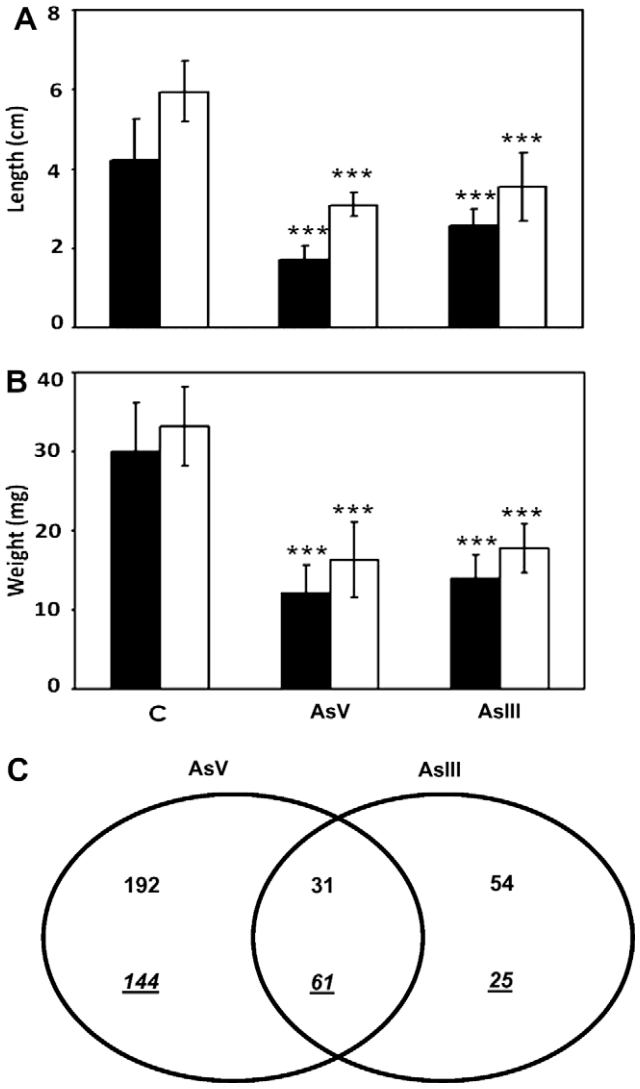

Fig. 1. Root (■) and shoot (□) length (A), root and shoot weight (B) of rice IR64 seedlings grown on AsV (250 μM) and AsIII (25 μM). Bars represent SD of means. *** indicate values that differ significantly from control at $P < 0.001$ according to Student's paired t-test. Venn diagram of the number of probe sets up- and downregulated in response to AsV and AsIII stress (C). The number of probe sets which were upregulated and the number of probe sets which were downregulated (italic) with an adjusted P value of <0.05 and a >2-fold change in gene expression.