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Research article

Genome-wide transcriptome profiling of genes associated with arsenate toxicity in an arsenic-tolerant rice mutant

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The presence of arsenic (As) in polluted environments, such as ground water, affects the accumulation of As in rice grains and causes a serious threat to human health. However, the precise molecular regulations related to As toxicity and tolerance in rice remain largely unknown. In the present study, we developed an arsenic-tolerant type 1 (ATT1) rice mutant by γ-irradiation mutagenesis and performed genome-wide transcriptome analysis for the characterization of As-responsive genes. Toxicity inhibited transcriptional regulation of putative genes involved in photosynthesis, mitochondrial electron transport, and lipid biosynthesis metabolism in wild-type (WT) and ATT1 rice mutant. However, many cysteine biosynthesis-related genes were significantly upregulated in both plants. We also attempted to elucidate the putative genes associated with As tolerance by comparing transcriptomes and identified ATT1-specific transcriptional regulation of genes involved in stress and RNA-protein synthesis. This analysis identified 50 genes that had DNA polymorphisms in upstream regions that differed from those in the exon regions, which suggested that genetic variations in the upstream regions might enhance As tolerance in the mutants. Therefore, the expression profiles of the genes evaluated in this study may improve understanding of the functional roles of As-related genes in response to As tolerance mechanisms and could potentially be used in molecular breeding to limit As accumulation in rice grains.

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1. Introduction

Metalloid arsenic (As) is ubiquitous in soil at low levels (Chen et al., 2017). Its presence in polluted environmental conditions such as ground water and cropping soil causes serious threats to human health (Joseph et al., 2015; Mohammed Abdul et al., 2015). Tens of millions of peoples in South and Southeast Asian countries such as Bangladesh, India, and China have been affected through contaminated drinking water and agricultural products such as rice (Rasool et al., 2015). Arsenic exists in both organic and inorganic forms, but the inorganic forms (arsenate (AsV) and arsenite (AsIII)) are more abundant in water and soil. However, methylated arsenic species (monomethylarsonic acid (MMAsV) and dimethylarsinic acid (DMAsV)) are also present in considerable amounts due to the accumulation of previously used methylated compounds as pesticides or herbicides (Lomax et al., 2012). The typical arsenic concentration varies from 0.01 to 3 μM in paddy field. However, fields irrigated with arsenic-laden ground water contained more than 33 μM As (Zhao et al., 2009; Panaullah et al., 2009). Rice is considered highly efficient in As accumulation in its grain compared with other cereals. The main As species present in rice grain and straw are inorganic arsenic (AsV, AsIII) and dimethylarsinic acid DMAv, although other methylated As compounds have also been found in rice (Sohn, 2014; Awasthi et al., 2017).

As uptake mechanisms are different between AsV and AsIII (Wang et al., 2011; Li et al., 2016). AsV is transported by roots through phosphate transporters whereas AsIII is transported through water channel aquaporins, mainly the nodulin 26-like intrinsic proteins (NIPs, a subfamily of the aquaporin family) with glycerol, ammonia, and silicic acid-like neutral solutes (Mukhopadhyay et al., 2014; Li et al., 2016). After AsV uptake through the roots, more than 90% of AsV is transformed into AsIII

Abbreviations: As, arsenic; AsV, arsenate; AsIII, Arsenite; H_2O_2, hydrogen peroxide; MMAsV, monomethylarsonic acid; DMAsV, dimethylarsinic acid; AR, Arsenate reductase; ROS, reactive oxygen species; GSH, reduced glutathione; GSSG, oxydised glutathione; ATT1, arsenic-tolerant type 1; WT, wild-type; PCs, phytochelatins.

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in the root and shoot tissues by enzymatic- or non-enzymatic pathways, and this reduction of AsV to AsIII is the initial stage of the plant As detoxification pathway (Finnegan and Chen, 2012). Transformation of arsenate to arsenite varies according to the plant species and plant parts. Previously, Raab et al., 2005 demonstrated the existence of 14 different arsenic species with the formation of different arsenite-phytochelatins (PCs) complexes (AsIII–PC_3, GS–AsIII–PC_2, AsIII–$(PC_2)_2$) and monomethylarsonic–PC2 (MA^{III}-PC2) in the roots of the arsenic non-tolerant sunflower plant, *Helianthus annuus*. In addition, accumulation and translocation of As in plant parts also depend on the organic or inorganic forms of arsenic exposed to plants. Mishra et al. (2017) identified the efficient reduction of As^V and MA^V to arsenite As^{III} and MA^{III} respectively in the root and shoot of rice plants but higher amounts of arsenic translocated to the shoot in methylated MA^v exposed plants compared with AS^v exposed plants in spite of the formation of several mixed complexes in the roots. Although, As detoxification occurs in plant cells, its accumulation can directly and/or indirectly affect plant metabolism such as photosynthesis (Srivastava et al., 2013), carbohydrate metabolism reactive oxygen species (ROS) and oxidative stress (Talukdar, 2013) and lipid peroxidation (Singh et al., 2011). Root is the first organ exposed to arsenic; therefore, both root elongation and proliferation are inhibited by arsenic toxicity (Norton et al., 2008). Moreover, arsenic toxicity interrupts biological processes including germination, shoot and root growth in plants. Arsenic tolerance by plants occurs through several cellular mechanisms, such as the release of toxic ions in the cell that sequester into internal organelles including vacuoles, inhibition of As transport, and As detoxification by phytochelatins (Li et al., 2016). Additionally, As exposure promotes the formation and accumulation of free radicals, ROS that causes the oxidative stress in plants (Birben et al., 2012). During As uptake by roots, AsV is generated by the imbalance of ROS production and is directly reduced to AsIII by antioxidant synthesis metabolism, which involves the GSSG transformation of glutathione (GSH) through arsenate reductase (AR) (Finnegan and Chen, 2012). Also, antioxidant synthesis confers GSH-mediated tolerance in plants during heavy metal stress (Cho et al., 2010) but the precise mechanisms of molecular regulations related to As toxicity and tolerance remain largely unknown.

Many previous studies have identified arsenic tolerance genes. They investigated the As tolerance mechanisms and quantified As accumulation in rice by developing transgenic and mutant lines. For example, Shri et al. (2014) suggested that As levels were higher in the shoots and roots of *Ceratophyllum demersum* PCS overexpressing transgenic rice line, $CdPCS_1$, but were significantly lower in the grain compared to the non-overexpressing line. In addition, overexpression of arsenic reductase genes in rice OsHAC1; 1 and OsHAC1; 2 significantly increased arsenite efflux, reduced As accumulation, and enhanced As tolerance in rice. Shi et al. (2016) showed that in aerobic soil, As accumulation in the rice grain was reduced in overexpressing plants, whereas As accumulation increased in the knockout mutant. More recently, a rice chloroquine resistance transporter (CRT)-like transporter mutant rice Osclt1 had a lower PC_2 level than WT, which led to reduce As accumulation in the roots, whereas levels remained the same or increased in the shoots (Yang et al., 2016). However, a genome-wide comparison of genes related to arsenate toxicity in different genotypes has been largely unreported. In this study, we selected As-tolerant rice mutants from TILLING populations induced by gamma ray mutagenesis, and performed a genome-wide transcriptome analysis of As-responsive genes to compare high-throughput gene expression profiles of wild type (WT) and As-tolerant rice mutants.

2. Materials and methods

2.1. Plant materials and growth conditions

M_7 rice mutant populations induced by gamma ray irradiation were developed according by Cho et al. (2010). As-tolerant rice mutants were selected by growing seeds of an M_7 rice population and a WT variety (cv. Donganbyeo) in soil under controlled conditions (16 h light/8 h dark cycle) at 25 °C for 2 weeks. In a preliminary experiment, WT seedlings were treated with AsV ($Na_2HAsO_4 \cdot 7H_2O$) As concentrations of 1500 or 2000 ppm for 3–5 days. The shoot and root growth was significantly affected after the 5-day As treatment at 2000 ppm (Fig. S1). Therefore, an As concentration of 2000 ppm was taken as severe As stress. Then seedlings produced by the M_7 TILLING lines were grown in the growth room under controlled conditions (16 h light/8 h dark cycle) at 25 °C for 2 weeks following treatment with 2000 ppm As, and the plant phenotypes were observed. We chose two As-tolerant rice mutant lines (ATT1 and ATT2) after the 2000 ppm As treatment and ATT1 was selected for further study because it had a stronger phenotype than ATT2. Finally, we treated each lineage with 150 ppm As for 5 days so that the phenotypic differences between WT and ATT1 could be compared. The morphological characteristics of the WT and ATT1 plants were also recorded (Fig. S2).

2.2. Determination of As concentration, detection of H_2O_2, and cell death

Two week-old seedlings of both plants were treated with AsV concentrations of 150 ppm for 3 d and then the treated seedlings were rinsed three times with distilled water. The rinsed plants were dried in a dry oven at 60 °C for 1 d and then shoot and root tissues were separately and then digested in 3:2 (v/v) HNO_3/H_2O_2 for 30 min using a microwave accelerated reaction system (MARS-X, HP-500 plus, CEM, USA). Total As species were determined using inductively coupled plasma mass spectrometry (ICP-MS, NexION 300D, Perkin Elmer, USA) with 3 independent biological replicates. For hydrogen peroxide (H_2O_2) detection, the AsV-treated plants were stained with 1 mg/ml 3,3′-diaminobenzidine solution (DAB; Sigma-Aldrich, ST. Louis, MA, USA) for 4 h and then incubated with a bleaching solution (ethanol: acetic acid: glycerol, 3:1:1). The incubated samples were boiled for 15 min at 95 °C and then visualized using electron microscopy. After 1 and 3 d of 150 ppm AsV treatment, we stained the two-week-old seedlings of WT and ATT1 with 0.025% [w/v] Evans Blue (Sigma-Aldrich, St. Louis, MO, USA) in distilled water for 30 min and washed the stained plants for 15 min with distilled water (Requejo and Tena, 2005). The roots stained with Evans Blue were observed under an operating microscope (Leica EZ4E, Diagnostic Instruments, Sterling Heights, MI) to estimate cell death.

2.3. RNA sequencing (RNA-seq) and transcriptome analysis

All samples of leaf and root tissues in AsV-treated plants were frozen in liquid nitrogen and ground to powder using a chilled mortar and pestle. Total RNA was isolated using Trizol reagent according to the manufacturer's recommended protocols (GibcoBRL, Cleveland, OH). For RNA sequencing, cDNA libraries were constructed from isolated RNA of each sample using the Illumina TruSeq kit according to Illumina's protocols and then the Illumina Hiseq2000 system was used for paired-end sequencing. To determine the proper mate inner distance of paired-end reads, the short reads were mapped against the Nipponbare reference genome sequence using the Burrows-Wheeler aligner (BWA; http://bio-bwa.sourceforge.net/), and the proper insert sizes of paired-end

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