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Research article

Transcriptome analysis of phytohormone, transporters and signaling pathways in response to vanadium stress in rice roots

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ABSTRACT

Trace concentrations of vanadium (V) have several benefits for plant growth, but high concentrations are toxic. To help characterize the cellular mechanisms underlying the toxic effects of V in plants, we present the first large-scale analysis of rice root responding to V during the early stages (1 and 3 h) of toxicity. Exposure to V triggered changes in the transcript levels of several genes related to cellular metabolic process, response to stimulus and transporters. Gene expression profiling revealed upregulated levels of genes associated with signaling and biosynthesis of auxin, abscisic acid (ABA) and jasmonic acid (JA) in V-treated rice roots. In addition, V upregulated the expression of ATP-dependent GSH-conjugated transport, ATP binding cassette (ABC) transporter, and markedly downregulated of the expression of divalent cation transporters, drug/metabolite transporter (DMT) and zinc–iron permease (ZIP). Among the V-specific responsive transcription factors and protein kinases, the most predominant families were NAC (NAM, ATAF, CUC) transcription factor, receptor-like cytoplasmic kinase VII (RLCK-VII) and leucine-rich repeat kinase VIII (LRR-VIII). These microarray data provide a new insight into the molecular mechanism of the rice roots response to V toxicity.

1. Introduction

Vanadium (V) is a metallic element present in most living organisms in trace amounts. V is important for the growth, yield and metabolic process of field crops. Foliar application of V to mature sugar beet (*Beta vulgaris* L.) increased the deposition of sucrose in the storage root [1]. Mild doses of V increased the yield of maize cobs significantly [2]. However, V is under scrutiny because the beneficial doses may also be toxic. Plants in soil with excessive fertilization of V showed decreased matter yield of shoots and roots. The relative root length, root surface area, root weight, and aerial dry weights of cuphea (*Cuphea viscosissima* × *Cuphea lanceolata* 'PSR 23') were decreased exponentially with increasing V concentration in hydroponic culture [3]. Also, V absorbed by rice plants greatly inhibited chlorophyll biosynthesis and interfered with sulfur-containing amino acids and crude protein, which resulted in decreased protein content [4].

Vanadium is one of the most potent known inhibitors of $Na^+ + K^+$ ATPase and may have a role as a physiologic regulator of the Na^+ pump [5,6]. Vanadium oligomers interact with several proteins and affect numerous biological mechanisms, such as membrane-bound transport systems, depending on the oligomeric species present [7]. Early studies showed that V caused G2/M cell-cycle arrest through reactive oxygen species (ROS)-mediated reactions [8]; it mimicked growth factor activities by inhibiting tyrosine phosphatases [9] and had inhibitory or stimulatory effects on the activities of receptor and non-receptor protein tyrosine kinases, depending on the oxidation state [10].

Rice (*Oryza sativa* L.) is one of the world's most widely grown grain crops and also a model plant for molecular biology research. Fertilizers (phosphate rocks and nitrogen, phosphorus and potassium fertilizer) contain high concentrations of V [11], and overdose of phosphate fertilizers may cause V accumulation in rice roots [12]. In our previous report, we found that excess V increased lipid peroxidation and markedly increased mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) activity in rice roots [13]. Here, we used whole-genome array to analyze the transcriptomic response to early V stress in rice root.

Abbreviations: ABA, abscisic acid; ABC, ATP binding cassette; CDPK, calcium-dependent protein kinase; DAB, 3,3′-diamninobenzidine; DMT, drug/metabolite transporter; Grx, glutaredoxin; GST, glutathione S-transferase; HMs, heavy metals; IRAK, interleukin-1 receptor-associated kinase; JA, jasmonic acid; LRR, leucine-rich repeat kinase; MAPK, mitogen-activated protein kinase; MDA, monodehydroascorbate reductase; NAC, NAM, ATAF, CUC transcription factor; NBT, nitroblue tetrazolium; RKF3, receptor-like kinase in flowers 3; RLCK, receptor-like cytoplasmic kinase; ROS, reactive oxygen species; Trx, thioredoxin; V, vanadium; ZIP, zinc–iron permease.

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We found genes involved in oxidation and its response to stimulus positively regulated with V stress. Hormone biosynthesis and signaling pathways also participated in this process. We further analyzed the expression of signaling, transcription factor and transporter genes with V treatments.

2. Results

2.1. Rice response to V

To determine the response of rice to V, we performed dose–response studies of root growth and found 1 mM V a suitable concentration for microarray experiments (Fig. 1A). Further increased V inhibited root elongation.

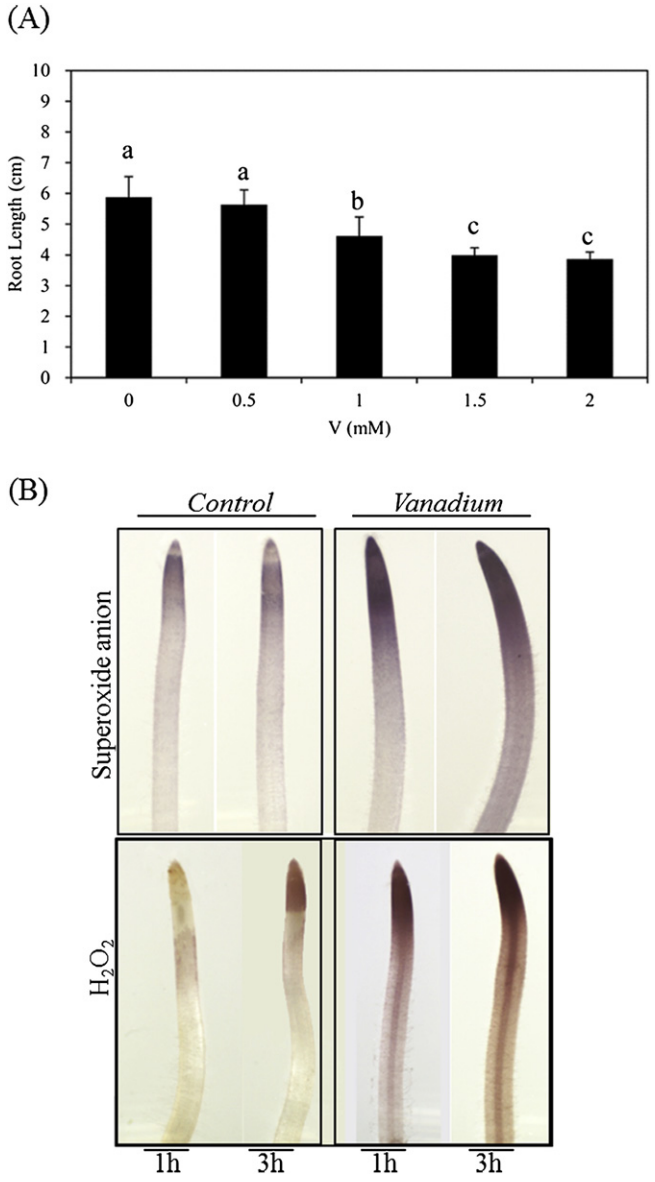

Fig. 1. The effect of vanadium (V) on rice root growth. (A) 6-day-old rice seedlings were treated with different concentrations of V for 3 days, and root length was measured. Different letters represent significant difference compared with 0 mM V, $P < 0.05$, ANOVA with Duncan's multiple-range test. (B) NBT (for superoxide anion) and DAB (for H_2O_2) were used to detect ROS generation in rice roots with 1 mM V treatment for 1 and 3 h. Water treatment was shown for control. Data are mean ± SD of 3 independent experiments.

2.2. Global expression profiles of rice root in response to V

To identify genes associated with the V response in rice root, we used large-scale expression profiling and found the expression of 1087 genes significantly upregulated and that of 281 genes significantly downregulated (Table S1). V-responsive genes were functionally classified into several categories by AgriGO functional enrichment analysis (Table S2). The major biological categories of the V-upregulated genes were cellular metabolic process, response to stimulus, localization and regulation of cellular process. For molecular function, the significant gene ontology terms were binding and transporter, catalytic, transferase and transcription regulator activity (Table 1).

2.3. ROS-related genes induced by V

Like many metals, treatment with vanadium generates ROS (Fig. 1B). We found 28 ROS network genes with significant signal intensity by our microarray assay (Table 2). These annotated rice transcripts belonged to class III peroxidase, monodehydroascorbate reductase (MDA), glutathione reductase, glutaredoxin (Grx) and thioredoxin (Trx). Transcripts for glutathione S-transferase (GST) also showed diverse responses to V stress. All of the V inducible GSTs were from the Tau family of GSTs.

2.4. Hormone-signaling genes regulated by V

V had a significant influence on the hormone biosynthesis and signaling pathway. In total, 33 hormone-responsive genes showed altered expression with V (Table 2, complete results see Table S3). Among them, 5 genes encoding auxin response transcription factors (OsIAA) were upregulated. Other phytohormones such as abscisic acid (ABA) and jasmonic acid (JA) showed increased expression in hormone signaling pathways. Five and 6 genes encoding for type 2C protein phosphatases and the jasmonate ZIM domain family, respectively, were upregulated.

2.5. Transporter genes regulated by V

Regulation of the membrane transporter system is an important component of metal stress responses. Several types of transporters showed differential expression with V treatment (Table S4). Genes encoding transporters for ATP-binding cassette-type and divalent cations were differentially regulated in the early (1 or 3 h) response to V (Fig. 2).

2.6. Transcription regulation and signal transduction under vanadium stress

We found 140 TFs representing 23 different families induced early with V treatment. The WRKY (17.2%) and NAC (11.5%) families were induced, then ZIM (5.7%), AUX/IAA (4.1%), ULT (0.8%) and MBF (0.8%) (Fig. 3A).

Nearly all of the V-responsive kinases on our microarray were associated with the interleukin-1 receptor-associated kinase (IRAK) group. The RLCK-VII (17.5%) and LRR-VIII (10.5%) families were significantly enriched, then RLCK-OS1 (5.3%), RLCK-II (1.8%) and RKF3 (1.8%) (Fig. 3B).

2.7. Semi-quantitative RT-PCR analysis of selected genes

To verify our microarray studies we analyzed RNA induction in response to V in the rice root by semi-quantitative RT-PCR. As shown in Fig. 4A, dose–response analysis revealed that induction of genes related to hormone signaling and transporter, and