DNA mismatch repair related gene expression as potential biomarkers to assess cadmium exposure in Arabidopsis seedlings

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In the current study, Arabidopsis seedlings were hydroponically grown on MS media containing cadmium (Cd) of 0.2–2.0 mg L\(^{-1}\) for 60 h of treatment. Gene expression profiles were used to relate exposure to Cd with some altered biological responses and/or specific growth effects. RT-PCR analysis was used to quantitate mRNA expression for seven genes known to be involved in DNA mismatch repair (MMR) system and cell division. Results indicated that Cd concentrations of 0.25–2.0 mg L\(^{-1}\) cause increased total soluble protein levels in shoots of Arabidopsis seedlings in an inverted U-shaped dose–response manner. Exposure to 0.25 and 0.5 mg L\(^{-1}\) of Cd dramatically induced expression of four genes (i.e. proliferating cell nuclear antigen 2 (atPCNA 2), MutL1 homolog (atMLH1), MutS 2 homolog (atMSH2) and atMSH3) and five genes (i.e. atPCNA1,2, atMLH1 and atMSH2,7), respectively, in shoots of Arabidopsis seedlings; Exposure to 1.0 mg L\(^{-1}\) of Cd significantly elevated expression of only two genes (atMSH6,7), but caused prominent inhibition in expression of three genes (atPCNA2, atMLH1 and atMSH3) in shoots of Arabidopsis seedlings. The expression alterations of the above genes were independent of any biological effects such as survival, fresh weight and chlorophyll level of shoots. However, shoots of Arabidopsis seedlings exposed to 2.0 mg L\(^{-1}\) of Cd exhibited statistically prominent repression in expression of these seven genes, and showed incipient reduction of fresh weight and chlorophyll level. This research provides data concerning sensitivity of expression profiles of atMLH1, atMSH2,3,6,7 and atPCNA1,2 genes in Arabidopsis seedlings to Cd exposure, as well as the potential use of these gene expression patterns as representative molecular biomarkers indicative of Cd exposure and related biological effects.

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1. Introduction

Cd is considered to be a highly toxic heavy metal element found with increasing frequency in the soil and water [1]. Its effects are not only physiological, but also genotoxic, including the broad spectrum of mutation such as point mutation and chromosomal aberrations which can even lead to immediate or stepwise cell death [2]. In the food chain, plants are ideally placed as early bioindicators of environmental pollution as they experience and respond to environmental toxicants earlier than organisms at higher trophic levels. The exposure of plants to Cd, even at low concentration, induces an increased production of reactive oxygen species, which results in several cellular stress responses and the unspecific damage to different cellular components such as membranes, proteins and DNA [1–3]. Therefore, the evaluation of the genotoxic effects of this heavy metal element on plants is an important topic in environmental studies [4].

According to Weeks [5], a “biomarker” is a biological response to a chemical (i.e. pesticides, heavy metals) which induces a stress and could indicate an exposure to a specific contamination and the biological effects related to that exposure. Biomarkers have been also defined as any observable and/or measurable variations at molecular, biochemical, cytological, physiological, biological, or behavioral levels revealing past or present exposure of an organism to one or several pollutants [6]. Sensitive biomarkers that detect early molecular-level changes leading to toxicity may improve our ability to detect sub-lethal effects of exposure and elucidate underlying mechanisms of contaminant-induced health effects [7]. Indeed, biomarker studies have now been incorporated into a number of national monitoring programs for human diseases. Due to the highly conserved nature of many genes, genes that serve as health biomarkers in Arabidopsis may also be relevant for predicting potential human health effects.

Since the response of cells in organisms to environmental stress or xenobiotic exposure involves altered gene expression, it is thought that biomarkers based on gene expression analysis might be a promising new approach [7–8]. Current investigations mainly focused on alterations of gene expression in animals and human.
being induced by low doses of heavy metals and organic pollutants, mainly in aquatic systems and in human diseases [6,9–13]. For example, Jelaso et al. [9] reported that exposure of developing Xenopus laevis tadpoles to low levels of PCB mixture A1254 (5–50 ppb) significantly increased expression of six genes such as p53 nerve growth factor (NGF), proopiomelanocortin, interleukin-1 converting enzyme, β-actin and glycerol-dehydrogenase independent of any health effects. Higher doses of A1254 (400–700 ppb) significantly decreased gene expression of NCF and β-actin that occurred concomitantly with decreased survival and the appearance of gross morphological and behavioral abnormalities in X. laevis tadpoles. These results indicated that increases in expression of specific genes can serve as biomarkers of exposure to A1254 and that decreases in expression of specific genes can serve as biomarkers of exposure and overt or impending health effects [9]. Similar observations were also reported in X. laevis frogs of chronic exposure to low dose of A1254 (24 ppm) in food [10]. Recent studies have revealed that the alterations in the expression of MMR genes in sporadic endometrial cancer may be a useful marker to predict the risk of colorectal cancer [12]. Examination of the literatures has enabled the identification of effectors present in most continental invertebrates in which gene expression could be affected by metallic pollutants. Liao and Freedman [14] showed in Caenorhabditis elegans (Nematode) the existence of a group of 32 genes which expression varied following Cd exposure. Accordingly, it has been demonstrated that only gene expression of catalase and metallothionein among the 14 effectors in Eisenia fetida could be considered as early biomarkers of exposure to Cd [6].

In all eukaryotes, post-replication DNA MMR is one of the principal DNA repair pathways that contributes to genomic stability by targeting base-base mismatches and insertion/deletion loops occurring during replication, homologous recombination and DNA damage [15–17]. It has been reported that there is accumulating evidence for interaction of the MMR system with various DNA lesions pointing to additional roles for mismatch repair. Recent studies have revealed that the expression patterns of MMR genes in human or microorganisms have been extensively used as an early biomarker particularly in relation to diagnosis of some human disease and pollution of aquatic environments [12]. Whilst there are some reports on alterations in gene expression in plants induced by adverse stress [18–21], it is still scarce for research on alterations in gene expression regarding cell division and MMR system in plants under pollution stress. Hays [16] suggested that highly conserved long patch MMR systems maintain genomic stability by correcting DNA post-replication errors, antagonizing homologous recombination, and responding to various DNA lesions. Plants encode a suite of MMR protein orthologs (i.e. MSH2, 3, 6, 7, MLH1 and PMS2) [16]. Accordingly, it has been demonstrated that proliferating cell nuclear antigen (PCNA)-binding proteins interact with many proteins such as MSH2, MSH6, MSH7 and MLH1, respectively [22]. Because higher plants are excellent materials to study the gene expression from these different viewpoints, Arabidopsis is of potential interest as sensitive model plant for addressing the environmental stress and/or pollution issues, and the genome study project of Arabidopsis has progressed well [15]. Our objective is to conduct laboratory-based studies on Arabidopsis seedlings and then follow this up with field testing in related plants. In the current research, semi-quantitative RT-PCR was used to study the expression signatures of MMR-related genes in shoots of Arabidopsis seedlings, and relate modifications in the gene expression with biological indexes and the seedling growth parameters measured under laboratory conditions. Such research is important in the broader context of verifying the genetic effects of Cd from contaminated land and water on the ecological environment and understanding of the relationships between environmental contamination and health risk, so as to propose appropriate remediation techniques with as few side-effects as possible.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of Arabidopsis thaliana (Arabidopsis, Columbia ecotype) were sterilized with 0.5% HgCl2 for 5 min, washed four to five times in sterile distilled water, and were imbibed in sterile water and placed at 4 °C for 72 h. Arabidopsis seeds were sown hydroponically in sterile flasks kept on a rotary shaker at 50 rpm (300 mL of flask containing 150 mL of Murashige-Skoog (MS) medium (Sigma–Aldrich) with 0.5% sucrose (pH 5.8)), at 15–20 plants per flask. The temperature was set at 21 ± 1 °C, the humidity at 60–70% relative humidity and the light intensity at approximately 3000 lx with a 14 h light and 10 h dark cycle. After the 7-day of incubation, the medium was supplemented with Cd at a final concentration of 0, 0.25, 0.5, 1.0 and 2.0 mg L⁻¹, respectively (in the form of CdCl2 2H2O of analytical grade with purity 99.5%, China) for 60 h. Each treatment had 3 replicates.

2.2. Determination of growth, chlorophyll and total soluble protein level of Arabidopsis shoots

After 60 h of Cd exposure, germination assay was made using seeds that had been simultaneously collected from every flask and then pooled. Germination rates were scored, and seedlings without at least 5 mm root length were considered as not having germinated. Plants were then harvested, and were washed carefully with sterile distilled water. Root length was determined by manual measurement of the main roots. The fresh weight of shoots in Arabidopsis seedlings was measured and then pooled to measure total soluble protein level following the standard lab protocol of Bradford with bovine serum albumin (RSA) as standard. Chlorophyll was extracted using 5 mL mixture of 50% acetone and 50% ethanol. Samples of 0.5 mg of Arabidopsis shoots were kept in the dark at room temperature of about 21 °C for 20–24 h, until the plant material was completely discolored. The extracts were centrifuged at 5000 × g and the absorption of the supernatant at optical density (OD) = 652 nm was measured. The following terms were used for calculation of chlorophyll (a+b) content:

$$\text{Chlorophyll (a+b) content} = \frac{\text{OD652} \times \text{extraction volume}}{34.5 \times \text{fresh weight}}$$

All pigment analysis was performed by 3 independent replicates.

2.3. Extraction of RNA and RT-PCR analysis

For both the control and Cd treatments, fresh shoot tissues were collected after 60 h of treatment in the growth chamber, and flash frozen in liquid nitrogen prior to storage at −80 °C. Total RNA was extracted from about 200 mg of fresh shoot tissue using Trizol Reagent as described by the Manufacturer’s Protocol. After total RNA isolation, the RNA was re-purified in order to remove possible genomic DNA by a DNase treatment (RNase-free DNase I, final concentration of 0.08 U µL⁻¹). The reaction was carried out at 37 °C for 30 min and RNA was extracted twice with phenol. RNA amount and purity (A260/A280 ratios) were determined fluorimetrically using the BioAnalyzer (Germany). Final integrity of total RNA was verified by electrophoresis of 1 µL total RNA on 1.0% agarose gels using DL2000 as a molecular weight marker ladder stained with ethidium bromide. The 5.0 µg of total RNA isolated from each treatment group was reverse-transcribed using Oligo- (dT) 15 Primers and the M-MLV reverse transcriptase into 20 µL cDNA following the protocol provided by the manufacturer. Chemicals used were obtained...
from the TaKaRa Biotechnology Ltd. (Dalian, China) unless otherwise mentioned.

Reverse transcriptase polymerase chain reaction (RT-PCR) amplifications and measurements were carried out in a thermocycler (Little Genius from PR China). Gene sequences used for primer design were obtained from http://www.arabidopsis.org. For each of the genes under study, a primer pair was designed to obtain a PCR amplification product (Table 1). The 5′ and 3′ halves of one of the oligo-nucleotides of each primer pair corresponded to the sequences of two exons flanking an intron, so that genomic DNA of the oligo-nucleotides of each primer pair corresponded to the expected number of PCR cycles required to be within the linear range of amplification at 94°C for 30 s) (Table 2), followed by a final 10-min extension at 72°C. Gene specific primers were tested to determine the optimal number of PCR cycles required to be within the linear range of amplification (Table 2). For each amplification, a negative control was run for each primer. Reaction mixtures were stored at 4°C until use. PCR products were run on 2.0% agarose gels in 1× TAE (Tris–Acetate–EDTA) buffer at about 100 V, and visualized by ethidium bromide staining. The image of each agarose gel was captured using a high-resolution scan of BioImane analyzer system (Venker, Italy), and the fluorescent intensity of each PCR product was measured using the bio-image analyzer system software Quantity One 4.2.3. Gene expression was normalized to the housekeeping gene 18S rRNA by taking the ratios of the gene band density to 18S of 18S rRNA are as follows: 18SF, 5′-AGTATGTCGCAAGCTGA-3′; 18SR, 5′-TGCTAATCTTACTATGTCG-3′. Primers for 18S rRNA were designed to have an annealing temperature of 58°C and yield 123 bp amplicons, respectively. All reactions were in triplicate.

2.4. Statistical analysis

All data are presented as means with standard deviation of the mean (SD). Mean values for indices tested were calculated from the results obtained for at least 3 replications. The Software SPSS for Windows (version 10.0) was used for the statistical evaluation of the results and plotting of the data. Statistical significance was determined by performing one-way analysis of variance (ANOVA) and comparisons of control and Cd-exposed groups were carried out using the least significant differences (LSD) test. Statistical differences were assigned at P < 0.05.

3. Experimental results

3.1. Effects of Cd on growth of Arabidopsis seedlings

The experiments were performed to evaluate the inhibitory effect of Cd stress on growth of Arabidopsis seedlings at all concentrations designed. Table 3 shows the shoots' fresh weight of the Arabidopsis plants grown with and without the Cd treatments after 60 h of exposure. Our results show that there were no statistically significant differences for fresh weight of shoots between the untreated control and treatment plants (Table 3, P > 0.05) although shoots at 2.0 mg L−1 of Cd showed incipient reduction of fresh weight. Likewise, Exposure to Cd of 0.25–2.0 mg L−1 for 60 h had no significant effect on the germination rate of seeds of Arabidopsis comparison to the control after 60 h of treatment (Table 3, P < 0.05). However, the differences between the root length of the control plants and the plants cultivated with Cd of 1.0 and 2.0 mg L−1 were found to be statistically significant (P < 0.05; See Table 3). Indeed, in seedlings exposed to 0.25–2.0 mg L−1 of Cd there existed a significant negative relationship between root length and Cd concentration \( r^2 = 0.956 \) (Table 3). This demonstrates a dose-dependent response.

3.2. Effects of Cd on total soluble protein and chlorophyll content of shoots in Arabidopsis

Fig. 1 shows the levels of total soluble protein and chlorophyll measured in the Arabidopsis shoot tissues depending on the concentration of Cd in liquid MS media following 60 h treatments. This figure shows that Cd induced an inverted U-shaped concentration response curve for the levels of total soluble protein in the shoot tissues. Low concentrations of Cd (0.25 and 0.5 mg L−1 in the liquid MS media) caused a significant increase in total soluble protein level relative to controls (P < 0.05, Fig. 1). The increase was 22% and 32%, respectively, in plants exposed to Cd of 0.25 and 0.5 mg L−1. Cd treatment of 1.0 mg L−1 had no significant effect on total soluble protein level relative to the controls although an increase in

### Table 1

<table>
<thead>
<tr>
<th>Target</th>
<th>Amplicon length (bp)</th>
<th>Sequence (5′ → 3′)</th>
<th>Tm (°C)</th>
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</thead>
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<tr>
<td>atPCNA1 F</td>
<td>126</td>
<td>GTG ACA CAG TTC TCA TCT GTG</td>
<td>60.9</td>
</tr>
<tr>
<td>atPCNA1 R</td>
<td>126</td>
<td>ATC ACA ATT GCA TCT GCC GG</td>
<td>61</td>
</tr>
<tr>
<td>atPCNA2 F</td>
<td>137</td>
<td>GAT GAA CTT CAT GCA TGAT CCA C</td>
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</tr>
<tr>
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<td>137</td>
<td>GAT GTC ATG ACG TTC TCA CGC</td>
<td>60.6</td>
</tr>
<tr>
<td>atMLH1 F</td>
<td>155</td>
<td>GTA GAG TCT TCT GTA AGC CA</td>
<td>61.5</td>
</tr>
</tbody>
</table>

### Table 2

<table>
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<tr>
<th>Ccd concentrations (mg L−1)</th>
<th>Genes</th>
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<tbody>
<tr>
<td></td>
<td>18S rRNA</td>
</tr>
<tr>
<td>0.25</td>
<td>18</td>
</tr>
<tr>
<td>0.5</td>
<td>17</td>
</tr>
<tr>
<td>1.0</td>
<td>18</td>
</tr>
<tr>
<td>2.0</td>
<td>17</td>
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</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Ccd level (mg L−1)</th>
<th>Root length (cm)</th>
<th>Inhibitory rate (%)</th>
<th>Fresh weight (mg/shoot)</th>
<th>Germination rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.9 ± 0.11</td>
<td>0</td>
<td>23.2 ± 0.14</td>
<td>89.5 ± 2.7</td>
</tr>
<tr>
<td>0.25</td>
<td>5.8 ± 0.29</td>
<td>1.7</td>
<td>23.6 ± 0.09</td>
<td>90.1 ± 3.3</td>
</tr>
<tr>
<td>0.5</td>
<td>5.1 ± 0.21</td>
<td>13.6</td>
<td>22.4 ± 0.10</td>
<td>90.9 ± 2.9</td>
</tr>
<tr>
<td>1.0</td>
<td>4.8 ± 0.18a</td>
<td>18.6</td>
<td>23.3 ± 0.12</td>
<td>88.9 ± 2.5</td>
</tr>
<tr>
<td>2.0</td>
<td>4.1 ± 0.09a</td>
<td>30.5</td>
<td>22.7 ± 0.08</td>
<td>91.2 ± 3.6</td>
</tr>
</tbody>
</table>

\( P < 0.05. \)
total soluble protein content in the shoots was measured as compared to the untreated controls (P<0.05, Fig. 1). In contrast, the levels of total soluble protein in the shoots at 2.0 mg L\(^{-1}\) of Cd substantially declined relative to controls (P<0.05, Fig. 1). In general, there was no substantial difference for Chlorophyll content in the shoots between control and Cd concentration of 0.25–2.0 mg L\(^{-1}\) following 60 h treatments (P<0.05, Fig. 1) although shoots exposed to 2.0 mg L\(^{-1}\) of Cd showed incipient reduction of chlorophyll level.

3.3. Effects of Cd on expression of MMR and PCNA genes in shoots of Arabidopsis seedlings

Fig. 2 shows expression profiles of MMR and PCNA genes in shoots of Arabidopsis plantlets hydroponically grown with and without Cd treatments for a period of 60 h. The following comparisons refer to specific Cd levels and the untreated controls (i.e. 0 Cd set to 100% max expression). Fig. 2 indicates that exposure of Arabidopsis to Cd concentration of 0.25 mg L\(^{-1}\) tested substantially up-regulated expression levels for the 4 genes, atPCNA2, atMSH2,3 and atMLH1 (P<0.05), but produced no apparent expression changes for atPCNA2, atMSH6, and atMSH7 genes as compared to the untreated control. Gene expression levels for atPCNA1, atPCNA2, atMLH1, atMSH2 and atMSH7 were also significantly elevated for Arabidopsis exposed to 0.5 mg L\(^{-1}\) Cd as compared to the untreated control (P<0.05, Fig. 2).

In the similar way, significantly enhanced expression levels for atMSH6 and atMSH7 genes occurred in plants exposed to Cd of 1.0 mg L\(^{-1}\), which dramatically repressed expression levels for atPCNA2, atMLH1 and atMSH3 genes, and which had no obvious effects on expression for atPCNA1 and atMSH2 genes, as compared to the untreated control. In contrast, shoots of Arabidopsis seedlings exposed to 2.0 mg L\(^{-1}\) of Cd exhibited statistically prominent decreases in expression of these seven genes (P<0.05, Fig. 2).

Overall, effect of Cd stress of different levels on expression changes of the above 7 genes in seedlings does not displayed a linear dose–response relationship; rather, it more closely follows the inverted U-shaped dose–response to Cd associated with hormesis (Fig. 2). These results agree with the previous results found in X. laevis frog of dietary exposure to Aroclor 1254 [10].

4. Discussion

Previous investigations showed the adverse effects of Cd stress on plants. These effects detected covered a wide range of metabolic changes including strong reductions in water and nutrient uptake, photosynthesis and leaf conductance [23], and gene expression in Arabidopsis [4], etc. Morphological changes such as growth inhibition, chlorosis and browning of root tips were demonstrated in several plants such as Scots pine [24]; oat and bean [25]; aquatic bryophyte [26]; garlic [1] and heterozy-gous tobacco (Nicotiana tabacum var. xanthi) and potato (Solanum tuberosum var. Korela) plants [27]. For example, root length was not repressed in transgenic Arabidopsis seedlings exposed to 25, 50 μM of Cd in agar MS, but was inhibited only in plants grown on medium containing 75–100 μM of Cd [4]. In our paper, the results indicated that a notable reduction of root length appeared only in plants exposed to Cd of 1.0 and 2.0 mg L\(^{-1}\) for 60 h of treatment. Otherwise, exposure to Cd of 0.25–2.0 mg L\(^{-1}\) did not significantly affect fresh weight or chlorophyll level in shoots (Fig. 1, Table 3), which is in agreement with the results of Zhang et al. [1]. Furthermore, small and similar retardation of shoot growth at higher concentrations (2, 5, 7, 10 mM) of CdCl\(_2\) was found in the study of Zhang et al. [1]. The reasons for the observed effects of Cd on, for example, fresh weight and chlorophyll level in shoots (Fig. 1, Table 3), could be traced to various factors, including intensity and duration of the exposure as well as composition of the growth medium. Moreover, the most important factor for elucidating the possible adverse effects of Cd is its actual accumulation in the plants. Numerous recent studies demonstrate that the accumulation of Cd is markedly higher in the roots than that in the shoots of Arabidopsis seedlings [28]. The role of thiol–rich phytochelatins in binding Cd and in accumulating it within vacuolar
compartments of root cells is responsible for the higher Cd content in roots [27]. This lower accumulation in the shoots may explain the lower toxicity of Cd observed in shoots of Arabidopsis seedlings in the present research (Table 3).

Many authors have demonstrated that expression of DNA MMR genes and PCNA genes can be altered in adverse circumstances. For example, Humbert et al. [29] found that increased expression of hMSH2 gene is driven by AP1-dependent regulation through phorbol-ester exposure. Mihaylova et al. [30] reported that 24–48 h exposure to hypoxic stress can reduce the expression of MLH1 and PMS2 genes in mammalian cells. Jensen et al. [31] demonstrated predictive value of MSH2 gene expression in colorectal cancer treated with capcetabnate. Kawakamia et al. [32] reported that MSH3 gene expression was down-regulated in bladder cancer and that LOH of MSH3 may be a more sensitive marker in bladder cancer. Recent studies have also revealed that the alterations in the expression of MMR genes in sporadic endometrial cancer may be a useful marker to predict the risk of colorectal cancer [12]. Thus, these results indicate that gene expression of MMR has a potential use for biomonitoring or diagnosing adverse stress. However, in plant species, the effects of adverse stress on gene expression of MMR remains a field that has to be explored. In the current experiment, the results indicate that Cd stress does affect expression patterns of MMR genes in shoots of Arabidopsis seedlings (Fig. 2). This suggests that Cd may have an adverse effect on the mismatch recognition process of the MutSα and MutSβ complex, and nucleotide excision process of the MMR system in Arabidopsis. Although the accurate timing and role of each gene product in the MMR complex has not been clearly elucidated, data shown in Fig. 2 indicate that the expression of different genes in the MMR system are affected differentially by the Cd exposure levels. For example, expression of four genes (i.e. atMLH1, atMSH2, 3 and atPCNA2) and of five genes (i.e. atPCNA1, 2, atMLH1 and atMSH2, 7) were prominently induced by exposure to 0.25 and 0.5 mg L−1 of Cd, respectively, for 60 h. However, exposure to 1.0 and 2.0 mg L−1 of Cd substantially down-regulated expression of two genes (atMSH 6, 7) and of seven yeast genes, tested, respectively (Fig. 2). Similar trend was reported on yeast species [33]. These authors found that exposure to low dose of Cd such as 5 μM affected the enzymatic activity of the MutSα and MutSβ complex in yeast and resulted in a significant decrease of ATP hydrolysis and DNA binding of the Msh 2–Msh6 complex but also of the Msh2–Msh3 complex. Thereby, modified expression of the MMR genes probably can be applied for biomonitoring Cd exposure.

Cd, like other adverse stresses, altered expression of DNA MMR genes in the Arabidopsis seedlings by RT-PCR analysis, and dose-dependent manners between Cd concentrations applied and expression of DNA MMR genes are inverted U-shaped curves with the maximum effect at Cd of 0.25 and 0.5 mg L−1 (Fig. 2), respectively, which is in agreement with the findings of previous report [10]. The major mechanistic explanations for induced expression of MMR genes observed by low doses of Cd in the current research are modulation of cellular signal transduction pathways by enhancement of protein phosphorylation and activation of transcription factors [29,34]. Low doses of Cd interfere with antioxidant defense systems and stimulate the production of highly reactive free radicals in cells [2–3]. Thus, these reactive free radicals in cells may act as signaling molecules and induce expression of DNA MMR genes in the Arabidopsis seedlings in this experiment (Fig. 2). Since the MSH2 protein has been implicated in the repair of oxidative DNA damage [15,29], induction of the atMSH4 genes could then represent a protective response to low doses of Cd in this research (Fig. 2). Alternatively, Cd has a high affinity to cysteine in three dimensional protein structures and can promote specific binding of Cd to MMR components [33,35], which can inhibit MMR gene expression exposed to 2.0 mg L−1 Cd (Fig. 2). Decreases in mRNA stability and increased mRNA turnover rates are other possible explanations for the observed changes in MMR gene expression (Fig. 2). Because a specific relation between Cd exposure and modified expression of certain MMR genes was observed (Fig. 2), generalized changes in mRNA transcription, stability and turnover seem less likely. All the Cd effects mentioned above undoubtedly would severely affect modifications in expression of MMR genes observed of Arabidopsis seedlings exposed to Cd in the current study (Fig. 2).

PCNA is an essential, ubiquitous, and highly conserved protein in eukaryotes that functions as the sliding clamp of DNA polymerase [22]. As a normal component of a multiple quaternary complex, PCNA plays a pivotal role in cell cycle regulation, DNA replication and repair of damaged DNA. Recent studies have revealed that PCNA is involved in interacting with many proteins such as MSH2, MSH6, MSH7 and MLH1, respectively [22,36]. Bishya et al. [37] reported that expression of PCNA gene was proportional with doses ranging 4–32 Gy of gamma irradiation. Moreover, expression of PCNA gene in rice seedlings was induced by exposure to a DNA-damage agent such as UV of 25 J m−2 and H2O2 of 1 mM treatment, indicating that the biomarker responses could be used to differentiate stress effect [22]. In this current research, we have found a notable induction of expression of atPCNA1 in shoots exposed to 0.5 mg L−1 Cd, and of atPCNA2 gene in shoots exposed to 0.25 and 0.5 mg L−1 Cd for 60 h, respectively, as compared to the control, suggesting that a minimal damage was required before the induction took place. In contrast, exposure to 1.0–2.0 and 2.0 mg L−1 of Cd significantly down-regulated expression of atPCNA2 and atPCNA1, respectively, independent of any observable effects on plant growth such as survival, fresh weight and chlorophyll level of shoots (Figs. 1 and 2, Table 3). These results suggest that modifications in expression of atPCNA1 and atPCNA2 in Arabidopsis can be used as molecular biomarkers indicative of Cd exposure.

The measure of molecular and population parameters exhibits several advantages because simultaneous application of a range of biomarkers is preferable to minimize misinterpretation of results and different biomarker responses could vary at different stages of the organism’s health status [38]. The soluble protein level in organisms, an important biomarker of reversible and irreversible variations in metabolism, is known to respond to a wide variety of stressors such as xenobiotics [39]. This biomarker is a reflection of the entire physiological state in the organism and a measure of its energy budget, which could be affected by chemically induced allocation of energy [40]. In this experiment, the significant induction in total soluble protein level of Arabidopsis shoots occurred after Cd exposure to 0.25–0.5 mg L−1 for 60 h as compared to the control (Fig. 1). The enhancement in protein level in organisms may also be a non-specific adaptive response induced by a variety of stresses including heavy metal contaminant stress [38]. In contrast, survival, shoot growth and chlorophyll content in Arabidopsis did not change substantially when Arabidopsis seedlings were exposed to 0.25–2.0 mg L−1 of Cd for 60 h (Fig. 1, Table 3). This indicates that these indexes were less sensitive to Cd than total soluble protein level. Moreover, apparent changes in total soluble protein level correlated well with significant changes in expression of atPCNA genes and atMSH2,3,6,7 genes (Figs. 1 and 2, Table 3), suggesting that changes in expression of atPCNA genes and atMSH2,3,6,7 genes, and total soluble protein level of Arabidopsis shoots can be used as biomarkers to assess the exposure of the organisms to Cd contaminant in the environment media.

The use of biomarkers to assess the exposure of the organisms to environmental contaminant has received increasing attention in the past decades. Biomarkers act as an integrative measurement at the suborganism level to indicate pollution status before harm to the biota occurs [41]. Indeed, biomarker studies have now been incorporated into a number of national monitoring programs.
for marine water [41]. Recently, use of gene expression profiles as potential biomarkers has been successfully utilized to detect various types of DNA damage and mutation in animals, bacteria and plants induced by adverse conditions in environmental monitoring [9–11,37,41–45]. In the field of toxicology, advances in transcript analysis have led to the recognition that altered gene expression is potentially early, rapid and sensitive molecular biomarkers indicative of pollutant exposure and impact. To our knowledge, available data about expression of MMR genes have focused mainly on human and microorganisms [29–32,46,47]. In this study, we have incorporated the use of RT-PCR method to determine MMR mRNA levels in Arabidopsis shoots after 60 h of Cd exposure. This method has some definite advantages over classic assays for DNA changes in cells of plants, i.e. the comet assay and micronucleus test, etc. [38]. The detection of MMR and PCNA at mRNA levels, in the current study, provide a direct measurement of their corresponding gene expression changes. The sensitivity of this assay also enables us to determine the induction of MMR and PCNA mRNA levels using as little as 0.05–0.1 g of fresh tissue samples. The RT-PCR method used in this study is not completely quantitative. The imperfection, however, could be compensated to some extent by using the one-tube, two-step RT-PCR method. In addition, the variability in PCR product output from one tube to another was reduced [41]. With the optimized cycle number and concentration of starting template, factors including limiting Taq polymerase and accumulation of inhibitors could be reduced [41]. Accordingly, it could produce better RT-PCR results. Nevertheless, the enhanced expression levels in DNA mismatch repair related genes among the controls and treatment groups, prior to the onset of significant health effects, were remarkable (Figs. 1 and 2, Table 3). The results clearly demonstrated the feasibility of using RT-PCR analysis of MMR and PCNA gene expression levels from Arabidopsis to assess environmental Cd contamination level and its remediation effects.

5. Conclusions

The present report defines modifications in MMR-related gene expression correlated with the induction of biological indexes and the seedling growth measured in Arabidopsis seedlings exposed to Cd for 60 h. We demonstrate that the inverted U-shaped dose–response effects of Cd stress on total soluble protein level in Arabidopsis shoots were observed, with maximum effects at 0.5 mg L−1 of Cd. The present study also demonstrated that the dose response curves for both atPCNA and MMR gene expression induced by Cd of 0.25–2.0 mg L−1 are inverted U-shaped curves with the maximum effect at 0.25 and 0.5 mg L−1 of Cd, respectively, which is in agreement with the findings of previous reports [10]. Four genes (i.e. atPCNA2, atMLH1, atMSH2,3, five genes (i.e. atPCNA1,2, atMLH1 and atMSH2,7), and two genes (atMSH6,7) in shoots of Arabidopsis seedlings after exposure to Cd of 0.25, 0.5 and 1.0 mg L−1, respectively, showed statistically substantial increases independent of observable health effects; However, after exposure to Cd of 2.0 mg L−1, shoots of the Arabidopsis seedlings showed significantly decreased expression level for the above seven genes that correlated with incipient decreases in fresh weight and chlorophyll level of Arabidopsis shoots. The ability to detect significant enhancement in expression of DNA mismatch repair related genes in Arabidopsis seedlings at Cd exposure levels of 0.25, 0.5 and 1.0 mg L−1, prior to the onset of significant health effects, would greatly improve environmental risk assessment. With further development in the future research, molecular tools may provide a more rapid and reliable means of establishing direct cause–and–effect relationships between Cd exposure and specific health effects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.jhazmat.2009.01.093.

References


