RESEARCH ARTICLE

PCR analysis of genes involved in base excision repair pathway in rice seedlings under Cr(III) exposure

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Abstract: The base excision repair (BER) pathway is an essential defense mechanism in plants against oxidative damage of DNA. Previous studies have reported that chromium (Cr) exposure causes oxidative stress and DNA damage due to accumulation of reactive oxygen species (ROS). In this study, hydroponic experiments were carried out to investigate expression of 21 candidate genes involved in BER pathway in rice seedlings exposed to Cr(III) using qRT-PCR analysis. Changes of H_2O_2 and $O_2^{-\cdot}$ content in rice tissues and the relative growth rate of rice seedlings were also determined. Results indicated that Cr(III) exposure caused dose-dependent inhibition on the relative growth rate of rice seedlings. H_2O_2 content in roots was significantly increased, while changes of H_2O_2 and $O_2^{-\cdot}$ content in shoots was consistent. PCR analysis showed that transcriptional changes of 21 selected candidate genes to Cr(III) exposure were tissue specific. BER pathway in roots was repressed by Cr(III) treatment but activated in shoots, suggesting that the BER pathway would play different roles in regulating and repairing DNA damage caused by Cr(III) exposure in different rice tissues.
Keywords: Cr, DNA, gene, PCR, ROS, rice seedlings

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Received: February 16, 2019; **Accepted:** March 15, 2019; **Published Online:** March 28, 2019
Citation: Ying-Chun Huang, Na Wang, Bi-Yong Huang, Chun-Jiao Lu, Xiao-Zhang Yu, 2019. PCR analysis of genes involved in base excision repair pathway in rice seedlings under Cr(III) exposure. *Applied Environmental Biotechnology*, 4(1): 30-36. http://doi.org/10.26789/AEB.2019.02.002

1 Introduction

Plant growth and development are detrimentally impacted by various biotic and abiotic factors, such as parasite, cold, heat, salinity and heavy metals (Tuteja et al., 2009; Yu and Feng, 2016; Yu et al., 2018a). Accumulation of reactive oxygen species (ROS) in plant cells is a typical reaction to different stresses (Fones et al., 2013; Tuteja et al., 2009). ROS mainly includes hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{-\cdot}$), singlet oxygen and hydroxyl radical ($OH^{\cdot}$), which are intrinsically formed during basic cellular metabolism at very low levels as a potential signal massager under favorable conditions (You and Chan, 2015). Un-expected enhancement of ROS in plant cells leads to negative effects on nucleic acids, membrane lipids and proteins (Bissenbaev et al., 2011), and even influences and causes cell homeostasis and/or DNA damage, eventually activates cell death programs and the BER pathway (Fones et al., 2013; Lu et al., 2017; Singh et al., 2009).

From an experiment, Georgiadou et al. (2018) reported that heavy metals caused ROS accumulation and cellular damage in plants. Previously, we also found that Cr exposure resulted in over-load of ROS in rice tissues (Yu et al., 2018b). Cr(III) is one of the most frequently detected species in the family of Cr. Cr exposure caused DNA damage, due to its strong binding tendency to sulfhydryl and carboxyl (Ramirez-Diaz et al., 2008), which further led to genotoxic stress and genomic instability (Tuteja et al., 2009). In fact, plants are able to trigger DNA repairing pathway to cope up with such damages (Beatriz et al., 2018). Mostly, DNA repair pathway includes base excision repair (BER), mismatch repair (MMR), nonhomologous end joining (NHEJ), nucleotide excision repair (NER) and homologous recombination (HR). The BER pathway is a primary DNA repairing channel, which fixes and removes damaged bases from the DNA chain through deamination, oxidation and alkylation in plants and mammals (Li et al., 2018). It was found that the BER pathway repaired DNA damage caused by Mn ions. Also, severe impact of Mn exposure on the BER pathway was problematic to yeast *S. cerevisiae* (Stephenson et al., 2013). Cd interfered and inhibited the BER pathway, which caused adverse effects on humans (Giaginis et al., 2006). Cr(VI) repressed expression of DNA repairing genes, leading to DNA damage in human bronchial epithelial cell line (16HBE cells) (Hu et al., 2018). To our knowledge, study of Cr(III) exposure on BER pathway in plants is still ambiguous. In this study, genetic responses of Cr(III) exposure to the BER pathway in rice seedlings were evaluated using qRT-PCR technique to analyze 21 candidate genes involved. Changes of H_2O_2

and $O_2^{-\cdot}$ content in rice tissues and seedling biomass were also measured to determine phytotoxicity of Cr(III) exposure. These works would be useful for better understanding the crosstalk of Cr(III) exposure with rice BER pathway due to accumulation of ROS in rice tissues.

2 Materials and Methods

2.1 Plant materials

Seedlings of rice (*Oryza sativa* L. cv. XZX 45) were prepared according to our previous work (Yu and Zhang, 2017). Rice seeds were planted in sandy soil under laboratory condition (Temperature 25C, Humidity 60%, Illumination intensity 7,000 lx) and irrigated with modified ISO 8692 nutrient medium. After 16 d of growth, rice seedlings were precollected, according to biomass size, and incubated in ISO 8692 nutrient medium for 12 h. After pre-treatment, rice seedlings were collected for the subsequent experiment.

2.2 Exposure regime of plants

Ten rice seedlings with similar size were exposed to 50 mL Cr(III) solution for 24 h. Four different Cr(III) treatments were conducted, namely 0, 12.0, 24.0 and 40.0 mg/L. Each treatment concentration was performed with four independent replicates. Chromium nitrate ($Cr(NO_3)\cdot 9H_2O$) (purity $\geqslant$ 99%) with analytical grade was used.

2.3 Measurement of relative growth rate

The relative growth rate of rice seedlings was determined as our previous study (Yu and Zhang, 2017), according to biomass changes of seedlings.

2.4 Analysis of H_2O_2 and $O_2^{-\cdot}$

Contents of H_2O_2 and $O_2^{-\cdot}$ in rice tissues were detected after Cr(III) exposure according to the methods of Li (2006).

2.5 Analysis of gene expression using qRTPCR

After exposing to Cr(III) solution (40 mg/L), rice seedlings were collected and divided into roots and shoots. Extraction of total RNA from treated and non-treated rice tissues was conducted as previously described (Yu et al., 2018a). Briefly, total RNA was extracted by Ultrapure RNA Kit (CWBIO, P.R. China), and the synthesis of cDNA was performed by the HiFiScript gDNA Removal cDNA Synthesis Kit (CWBIO, P.R. China). qRT-PCR was determined by a 7500 Fast Real-Time PCR system (Applied Biosystems) with the UltraSYBR Mixture (CWBIO, P.R. China). The OsGAPDH1 (glyceraldehyde-3-phosphate dehydrogenase, LOC_Os08g03290.1) was selected as the internal control (Yu et al., 2018b). Primers sequences used are listed in Table 1. The relative expression of each tested genes was determined by the $2^{\Delta\Delta\ CT}$ method (Livak and Schmittgen, 2011). All data were referred to mean of 4 independent biological replicates SD.

Table 1. Sequences of primers used in this study

Gene name	Locus identifier	Primer sequence (5'-3')	Product length (bp)
osgapdh1	LOC_Os08g03290.1	F-GACAGCAGGTCGAGCATCTTC R-CAGGCGACAAGCTTGACAAAG	74
osogg1	LOC_Os02g34750.1	F-CTGGCAAGAGCTTAACCCCA R-TCTGTGCCCAACCAGCATAG	87
osnth	LOC_Os06g13070.1	F-CAAAGACTGTGGCGTGTGTG R-CTAGCTGTTGCAGGAACCCA	112
osnth- like1	LOC_Os11g16580.1	F-CCAAAGGATGAGTGGGAACC R-GAAAGCTGAAGGGCAGATGT	120
osape2	LOC_Os09g36530.1	F-ACAACTCCAGCAACCGAAGA R-GAGCACGGGCACAGACATAG	214
osxrcc1	LOC_Os06g05190.1	F-GGATGGGGTGGTCTTTGTA R-AGTTTTCTTTGGCTGTGAG	225
osung	LOC_Os04g57730.1	F-GTGAGAGAACATCAAGCC R-TCCCCAGAGAATAAAGAC	114
osmuty	LOC_Os12g10850.1	F-ACGGTGAAGAGATTCTGGCA R-TGGCTTTTGGGACTACTCGT	220
osmuty- like	LOC_Os12g10900.1	F-GGAGGTGAACGAGATGTGGG R-AATCCCCAATGCCACGAACT	140
osmpg	LOC_Os02g53430.1	F-AGGACTGCTCCTGTGTTTGG R-GCTCCAACTCCCTCCTTGTC	104
osmbd4	LOC_Os09g01290.2	F-AAGCGAAAGAGTAGGGAG R-AGCAGAACTGGTCAAAGA	346
ospcna	LOC_Os02g56130.1	F-CGGTGTCACTAACCTTTGC R-CACGACTTCATTTCCTCAT	187
ospolδ3	LOC_Os01g10690.1	F-GCTTGTGAGTTCATTTCCG R-TGAGCCTTGTTTTTGTTTC	249
ospolδ2	LOC_Os03g03650.1	F-GCCACATCTACTTCACGC R-AATAGCAGACCTCTCCTT	200
ospolδ4	LOC_Os08g06620.1	F-GCCGCGACAATCCATAGAGA R-CTTTCTGGAAGCGGATCGGT	458
ospolδ4A	LOC_Os09g34850.1	F-AAAGGAGGTCCCACCAAAG R-AGACGAGTAACGCCAATGC	215
ospolδ1	LOC_Os11g08330.1	F-TACTTTGGTCCCCCCTG R-CTGCTTTCGCCCTTTTC	161
ospolε1	LOC_Os02g30800.1	F-GTGGTTGTCAATCCGTTC R-ATAGTTGTTATCCCGTGC	306
ospolε2	LOC_Os05g06840.1	F-ATTGTAGCAGAAGGGGA R-CAGGACCAGGAATGAAC	445
ospolε3	LOC_Os09g39490.1	F-TTTGCGGTGTAAACGCTGTG R-CAACTGATGAAGCCGCCAAG	148
osfen1	LOC_Os05g46270.1	F-TGCCACCAAAGAACTAACAG R-CATCAAATGACGAAGAAACC	268
oslig1	LOC_Os10g34750.1	F-GTTAGCAGAAAAAACCGT R-AACATAGGACCAACAGGA	234

2.6 Statistical analysis

Analysis of variance (ANOVA) and Tukeys multiple range tests were used to determine the statistical significance at 0.01 or 0.05 between treatments (Zar, 1999).

3 Results

3.1 Relative growth rate of rice seedlings exposed to Cr(III)

Compared with untreated seedlings, relative growth rates of rice seedlings showed a downward trend after Cr(III) exposure for 24 h (Fig. 1). Relative growth rates of rice seedlings decreased to 9.79% at 12.0 mg Cr/L, and then declined dramatically to approximately 2.99% by 40.0 mg Cr/L. Overall, relative growth rate was significantly declined with enhancing Cr(III) concentrations.

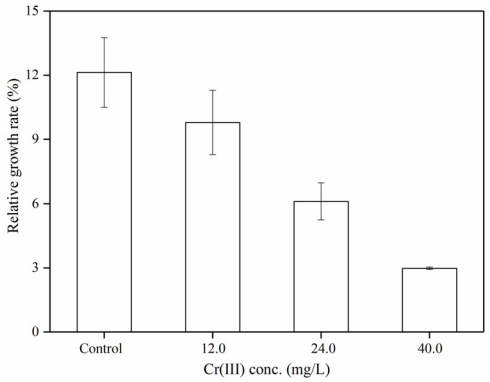

Figure 1. Changes of relative growth rate (%) of rice seedlings exposed to Cr(III) for 24 h. Data refer to the mean ± SD of four replicate samples. Vertical lines represent SD.

3.2 Change of H_2O_2 and $O_2^{-\cdot}$ content in rice seedlings exposed to Cr(III)

(Fig. 2a & 2b) showed the changes of H_2O_2 and $O_2^{-\cdot}$ content in different tissues (roots/shoots) after 24 h period of Cr(III) exposure. Responses of H_2O_2 content in rice tissues showed differently (Fig. 2a). Compared with control shoots, H_2O_2 content in shoots exposed to 40.0 mg/L increased to approximately 1.38-fold. However, much more changes of H_2O_2 content in roots were detected, which is approximately 3.01-fold higher than control. Changes of $O_2^{-\cdot}$ content in both shoots and roots to Cr(III) exposure were identical, where the fold changes of $O_2^{-\cdot}$ content were determined to be 1.42 and 1.37 in shoots and roots, compared with controls, respectively (Fig. 2b).

3.3 Expression of genes involved in BER pathway in rice seedlings exposed to Cr(III)

Twenty-one genes were identified in rice BER pathway using the online program of KEGG (https://www.kegg.jp/) (Fig. 3). In shoots, almost all identified genes showed positive expression after Cr(III) exposure, but only 13 genes were significantly up-regulated ($p< 0.05$) (Fig. 4a). In roots, 21 genes was down-regulated, but 18 genes were significantly repressed by Cr(III) exposure ($p<0.05$) (Fig. 4b).

4 Discussion

Previous studies reported that Cr(III) exposure caused over-generation of ROS in plant cells, leading to oxidative stress (Balamurugan et al., 2002; Wang et al., 2006b), which eventually resulted in DNA lesions in mammalian (Hashiguchi et al., 2004). It was found that Cr(VI) exposure induced DNA damage in mammalian cells, which was repaired by BER pathway (Bryant et al., 2006). However, many heavy metals inhibited DNA repair through the BER pathway in 293T cells (Wilson et al., 2004). It was found that Cr(VI) exposure significantly increased ROS content in tall fescue (Huang et al., 2018). The mustard treated with 20 μM Cr(VI) also showed significant increases of H_2O_2 in tissues, where more H_2O_2 accumulation was detected in roots rather than leaf (Pandey et al., 2005). In this study, Cr(III) exposure affects and/or inhibits the biomass growth of rice seedlings, and caused accumulation H_2O_2 and $O_2^{-\cdot}$ in rice tissues. PCR analysis revealed that expression of 21 selected candidate genes involved in rice BER pathway were inconsistent in rice tissues (roots/shoots), suggesting their different responsive and repairing strategies between roots and shoots of rice seedlings after Cr(III) exposure. This is most likely due to the fact that there are significant differences in speciation, localization and subcellular distribution of Cr(III) in rice seedlings between shoots and roots (Yu and Feng, 2016).

It is known that BER pathway, including short-patch repair (SP) and long-patch repair (LP), plays an important role in cellular defense against DNA damage (Cordoba-Canero et al., 2009). The first step in BER pathway is to recognize and remove damaged bases by various types of glycosylases, namely 8-oxo-G repair glycosylases OGG1 (Excision of 8-oxo-G), MYH (MUTY homolog, Excision of A mispaired with 8-oxo-G), oxidized pyrimidine glycosylases NEIL1-3 (Excision of oxidized pyrimidines), nth (Excision of oxidized pyrimidines), uracil/thymine glycosylases UNG (Excision of uracil), MBD4 (Binds methylated DNA and excision of 5-hydroxymethyluracil), TDG (Excision of mismatched thymines and uracils), SMUG1 (Excision of uracil) and Methyl-purine glycosylases MPG (Excision of methyladenine and methylguanine) (Kurthkoti et al., 2010; Sharbeen et al., 2015). The second step involved in BER pathway is cleavage of the sugar-phosphate backbone of DNA at the site where the damaged base was removed by specific glycosylase (Murphy et al., 2009). As shown in Fig. 4a, relevant genes of osogg1 and osape2 were expressed differentially in different tissues of rice seedings under Cr(III) exposure. In fact, expression patterns of *osogg1*, *osnth*, *osnth*-like1, *osung*, *osmuty*, *osmuty*-like, *osmpg*, *osmbd4* and *osape2* genes in shoots generally showed upward trend, of which *osnth*, *osmuty* and *osmbd4* were significantly increased ($p<0.05$). It has been reported that in Arabidopsis *nth* gene played a major role in DNA

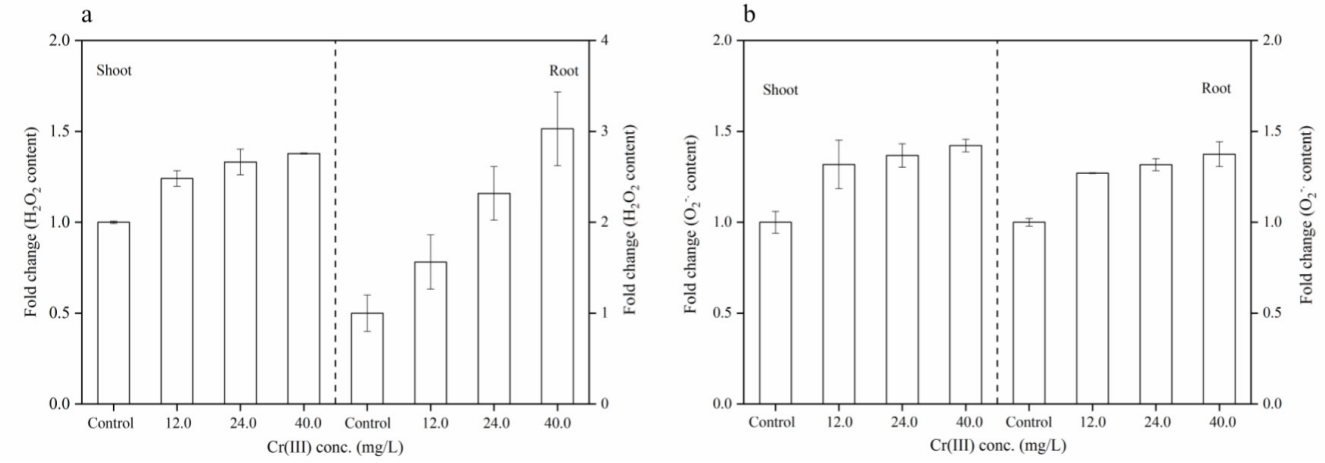

Figure 2. Fold change of H_2O_2 (a) and $O_2^{\cdot-}$ (b) content in rice seedlings exposed to Cr(III) for 24 h. Data refer to the mean ± SD of four replicate samples. Vertical lines represent SD. *$p<0.05$ indicated significant difference between the exposed group and control.

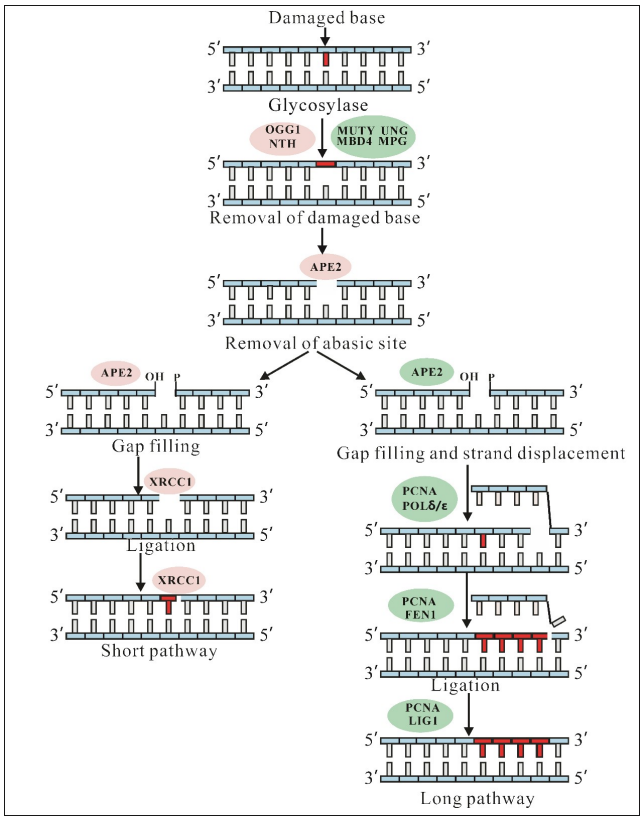

Figure 3. Schematic illustration for the pathway of base excision repair (BER) in plants. The pathway was taken from the database KEGG (https://www.kegg.jp/kegg/pathway.html). Green color indicated the defined genes functioning in the rice LP BER pathway, while pink color refers to the defined genes involved in the rice SP BER pathway.

repair against DNA damage (Roldanarjona et al., 2000). Overexpression of gene mbd4l in Arabidopsis stimulated BER pathway and had a positive effect on repairing DNA damage caused by oxidative stress (Nota et al., 2015). In roots (Fig. 4b), Cr(III) exposure caused remarkable down-regulation of osogg1, *osnth*, *osnth*-like1, *osung*, *osmuty*, *osmuty*-like, *osmpg*, *osmbd4* and *osape2* genes ($p<0.05$).

It is noticed that the coming step in SP of BER pathway is to fill up the gap by XRCC1 (X-ray repair cross-complementing protein 1) and DNA polymerase (*polβ*). However, a different repairing procedure has been found in LP of BER pathway, in which DNA polymerases, such as *polδ*, *polε*, *polβ* and *PCNA*, are mainly responsible for filling up the gap and replacing the strand (Sokhansanj et al., 2002). In this study, after Cr(III) exposure, expression of *osxrcc1* and *ospcna* genes in shoots showed positive responses, but negative expression in roots. *xrcc1* is an important component in the BER pathway in plants, functioning in DNA repair against oxidative damage (Martinez-Macias et al., 2013). *PCNA* is also involved in DNA repair or replication and induced activity of *POLδ* (Strzalka and Ziemienowicz, 2011). It has been reported that *POLδ* was important for DNA repair, and *POLε* played a role in maintaining genomic stability (Nicolas et al., 2016; Okimoto et al., 2016). It is evident that dysfunction of *POLδ* led to genomic instability (Zhang et al., 2016). In this study, expression of *ospolδ* and *ospolε* genes (*ospolδ1*, *ospolδ2*, *ospolδ3*, *ospolδ4*, *ospolδ4a*, *ospolε1*, *ospolε2*, *ospolε3*) in shoots were up-regulated, of which *ospolδ1*, *ospolδ2*, *ospolδ3*, *ospolε1*, *ospolε2* and *ospolε3* showed significant up-regulation compared with control ($p<0.05$). In roots, expression levels of all *ospolδ* and *ospolε* genes were significantly down-regulated ($p<0.05$), except for *ospolδ3* and *ospolε3*.

In LP BER, an additional step is to cleave the incorrect

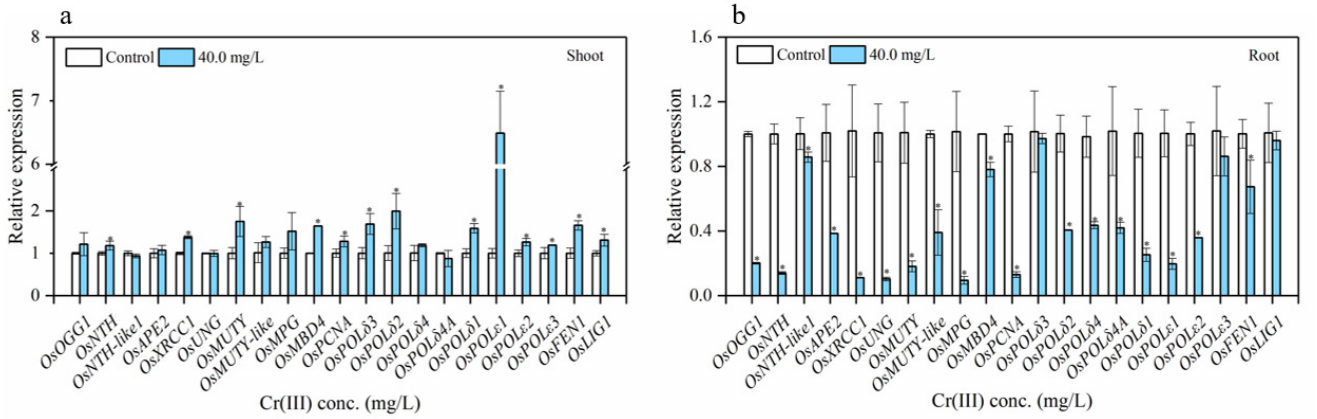

Figure 4. Expression analysis of 21 candidate genes in shoots (a) and roots (b) of rice seedlings exposed to 40 mg Cr(III)/L for 24 h. Data were the mean SD of four replicate samples. Vertical lines represent SD. *$p<0.05$ indicated significant difference between the exposed group and control.

DNA bases by flap-endonuclease (*FEN1*) and *PCNA* (Sharbeen et al., 2015; Sokhansanj et al., 2002). In our results, significant up-regulation of *osfen1* gene and *ospcna* in shoots was detected, where in roots it was contrary. Deficiency of *fen1* in plant cells led to hypersensitivity to H_2O_2 accumulation in vertebrate cells (Asagoshi et al., 2010). The final step is to seal the DNA ends. In SP of BER pathway, it was completed by the DNA ligase 3 (*lig3*) and *xrcc1*, where the DNA ligase 1 (*lig1*) and *pcna* played a central role in LP BER. In this study, all genes (*oslig1*, *ospcna* and *osxrcc1*) involved in the final step of BER pathway were up-regulated significantly in shoots. However, in roots expression of all genes was negative. Overall, the transcription patterns of candidate genes involved in BER pathway were opposite between shoots and roots.

It is evident that heavy metal directly or indirectly induced ROS production, and led to genotoxicity in vivo, for example, inhibiting DNA repair and causing gene abnormalities (Wang et al., 2006a; Yuan et al., 2016). Cd at high concentrations (>50 μM) caused necrosis and repressed DNA repair (Giaginis et al., 2006), where Cr(VI) also inhibited DNA repair in mammalian cells (Hu et al., 2018; Templeton and Liu, 2010). After 24 h of Cr(III) exposure, down-regulation of genes involved in BER pathway were observed in roots, indicating inhibition of BER pathway. This is most likely due to the fact that over accumulation of Cr(III) in rice roots affected the enzymes of DNA repair and led to genotoxicity. However, in shoots, after Cr(III) exposure, the genes responsible for BER pathway were all up-regulated. It means that BER pathway against DNA damage was activated due to Cr(III) exposure in rice seedlings.

Our findings demonstrated that Cr(III) inhibited the growth of rice seedlings, and caused accumulation of H_2O_2 and $O_2^{-\cdot}$ in rice tissues. PCR analysis of gene transcription reveled that down-regulation of genes resulted from over-accumulation of ROS in roots as well as metal ions binding with specific protein, suggesting inhibition of Cr(III) exposure on BER pathway in roots. In shoots, BER pathway was activated by less accumulation of metal ions and ROS, and subsequently stimulated the plant DNA damage repairing system. Overall, different responsiveness and regulation mechanisms existed between roots and shoots of rice seedlings during repairing of DNA damage caused by Cr(III) exposure through BER pathway.

Author Contributions

Ying-Chun Huang, Na Wang, and Bi-Yong Huang performed experiments and collected data. Chun-Jiao Lu conducted data analysis and drafted manuscript. Prof. Dr. Xiao-Zhang Yu conceived the study and finalized the submission. All authors approved the final manuscript.

Conflict of Interest and Funding

The authors declare no conflict of interest. This work was financially supported by the Natural Science Foundation of Guangxi (No: 2018GXNSFDA281024) and the College Students Innovation and Entrepreneurship Training Program (2018) of Guilin University of Technology (No: 201810596361).

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