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Role of hydrogen sulfide in the methyl jasmonate response to cadmium stress in foxtail millet

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1. ABSTRACT

Methyl jasmonate (MeJA), a vital cellular regulator, mediates diverse developmental processes and defense responses against environmental stresse. Recently, a novel gasotransmitter, hydrogen sulfide (H_2S), was found to have similar functions, but the interactions between H_2S and MeJA in the acquisition of cadmium (Cd) tolerance have not been reported. Treating foxtail millet with 1 microM MeJA not only enhanced Cd tolerance and alleviated growth inhibitions but also decreased the contents of hydrogen peroxide, malondialdehyde and Cd in seedlings under 200 microM of Cd stress. Exogenous application of MeJA inhibited the transcript levels of the Natural Resistance-Associated Macrophage Protein (*NRAMP1* and *NRAMP6*) and intensified Cd-induced expression of the homeostasis-related genes (*MTP1*, *MTP12*, *CAX2* and *ZIP4*, besides *HMA3*). In addition, treatment with MeJA induced the production of endogenous H_2S. Fumigation with sodium hydrosulfide (H_2S donor) significantly enhanced MeJA-induced Cd tolerance, but this ability was weakened when H_2S biosynthesis was inhibited with hydroxylamine. These results suggest that pretreatment with MeJA alleviated Cd stress and that this improvement was mediated by H_2S in foxtail millet.

2. INTRODUCTION

Hydrogen sulfide (H_2S) is a colorless gas that can be toxic to living organisms. After nitric oxide (NO) and carbon monoxide, H_2S was reported as the third signaling gasotransmitter (1). In humans, H_2S plays various roles in different systems, including blood flow, neurotransmission, immune reactions, hormone secretion and muscle contraction (1, 2). H_2S is mainly synthesized by cystathionine ß-synthase (EC 4.2.1.2.2.) and cystathionine γ-lyase (EC 4.4.1.1.) in mammals, while its formation is mostly catalyzed by L-cysteine desulfhydrase (EC 4.4.1.1.), D-cysteine desulfhydrase (EC 4.4.1.1.5.) and ß-cyanoalanine synthase (EC 4.4.1.9.) in plants (2-4). In addition, *O*-acetylserine(thiol)lyase, a cysteine synthase-like protein, also possesses cysteine desulfhydrase activity (5). In general, aminooxyacetic acid (AOA), potassium pyruvate (PP) and hydroxylamine (HA) are used as H_2S biosynthesis inhibitors and hypotaurine (HT) is used as a H_2S scavenger (6, 7). Recent studies have indicated that H_2S has extensive regulatory functions in physiological and biochemical processes (4). In the growth and development of plants, H_2S participates in seed germination (8), photosynthesis (9), lateral root formation (10), flower senescence (11) and stomatal movement (12). H_2S can also alleviate various abiotic

stresses, such as drought, heat, cold, osmosis, salt and heavy metals (4, 7, 13-15). Our previous study showed that H_2S might be a critical component in abscisic acid (ABA)-induced stomatal regulation by ion channels in *Arabidopsis* (13). H_2S also interacts with other phytohormones, including auxin, gibberellic acid and salicylic acid (SA) (6, 10, 16). Although there have been several studies regarding the interactions between H_2S and methyl jasmonate (MeJA), they are still unclear.

MeJA and jasmonic acid (JA), members of the cyclopentanone compound family, which are synthesized from linolenic acid via the octadecanoic pathway, exhibit signaling functions and are cellular regulators for defense responses against biotic and abiotic stresses (17). MeJA could alleviate plant damage, inhibit metal uptake and enhance the antioxidative capacities in plants (18-20). Cadmium is a typical poisonous metal for plants, and excessive uptake of Cd not only damages photosynthesis, root elongation and enzymatic systems but also induces or inhibits gene expression, disrupts normal homeostasis of essential metals, and alters ultrastructures (21, 22). Therefore, it is worthwhile and necessary to investigate a Cd detoxification strategy in cereal crops. Treatment with sodium hydrosulfide (NaHS, H_2S donor) enhanced Cd tolerance in rice and wheat seedlings (23, 24). However, to the best of our knowledge, a cross between MeJA and H_2S in the improvement of Cd tolerance in foxtail millet (*Setaria italica*) seedlings has not yet been reported.

Foxtail millet is the second most widely planted species of millet in arid and semi-arid regions of Asia and Africa and is known for its drought-tolerance, dense root system, small genome, and low repetitive DNA content. As a result, it may represent an appropriate model for studying crop species (25). Cd in soil affects crop yield and grain quality; therefore, there is an urgent need to find a novel alleviation strategy for Cd stress. Based on the above-mentioned studies, we hypothesized that the interaction between MeJA and H_2S might enhance Cd tolerance in foxtail millet seedlings. In this study, we investigated the roles of H_2S on MeJA responding to Cd stress through changes in growth status, reactive oxygen species (ROS), Cd accumulation, and expression levels of several genes of metal transporters in foxtail millet roots.

3. MATERIALS AND METHODS

3.1. Plant materials and treatments

Seeds of foxtail millet cultivar, 'Jingu-21', were from a local market (bred from the cash crops research institute, Shanxi Academy of Agricultural Sciences, Shanxi Province, China). Seeds were sown and grown in pots containing perlite and vermiculite (1:3, v/v) in growth chambers at 23°C, with a cycle of 16 h of 160 μE $m^{-2}s^{-1}$ illumination and 8 h of dark at a 60% relative humidity. Our pre-experiments revealed that treatment with a 200 μM cadmium chloride ($CdCl_2$, Sigma-Aldrich, Shanghai) solution could inhibit seedling growth. Therefore, a 200 μM $CdCl_2$ solution was used to treat the seedlings. After germination, seedlings were treated according to the following descriptions: 1) control check (Ck), 2) Cd, 3) MeJA+Cd, 4) H_2S+Cd, 5) H_2S+MeJA+Cd, and 6) HA+MeJA+Cd. All of the agents (NaHS, AOA, PP, HA, HT, and MeJA, Sigma-Aldrich, Shanghai) used in this study were of analytical pure (A.P.) grade. Thirty plants per pot were arranged by the different treatments in the growth chamber with three replicates for each treatment.

3.2. Determination of H_2S content

The endogenous H_2S content was determined using a Four-channel Free Radical Analyzer instrument (TBR4100 WPI, Sarasota, FL, USA). The seedlings (200 mg) were homogenized with 2 mL of extraction buffer (50 mM phosphate buffer, pH 6.8., 0.2. M ascorbic acid, and 0.1. M ethylene diamine tetraacetic acid (EDTA)) (7), and then the H_2S content in the tissue homogenate was determined using tissue electrodes. NaHS was used in the available H_2S standard curve measurement and all of the steps were in accordance with the instruction manual.

3.3. Histochemical detection of hydrogen peroxide (H_2O_2) and the superoxide (O_2^-) anion in the roots

The histochemical detection of H_2O_2 and the O_2^- anion used 3,3′-diaminobenzidine (DAB) and nitrotetrazolium blue chloride (NBT) as chromogenic substrates, respectively. The determination assay was performed as described in Kumar *et al*. (2014) (26). After staining with DAB or NBT, the roots (~2 cm long) were extensively washed with distilled water and photographed on color film (EOS 70D, Canon Photo Film, Tokyo, Japan).

3.4. Histochemical localization of lipid peroxidation and determination of the malondialdehyde (MDA) content in the roots

The histochemical detection of lipid peroxidation was performed with Schiff's reagent as described by Pompella *et al*. (1987) (27). The stained roots were extensively washed and then photographed on color film (EOS 70D). The MDA content was expressed in nmol g^{-1} (fresh weight) according to the previously described method (28). Roots were weighed and homogenized with 5% trichloroacetic acid (TCA), and then, an equal amount of 0.6.7% 2-thiobarbituric acid (TBA) was added to the supernatant after centrifugation at 1,662 ×*g* for 5 min at 20°C, followed by boiling the mixture at 100°C for 30 min. After cooling to room temperature and centrifugation at 11,238 ×*g* for 10 min, the absorbance at 450, 532 and 600 nm was measured.

3.5. Analysis of mRNA levels

The total RNA of the roots was extracted with TRIzol Reagent (TaKaRa, Tokyo, Japan) and cDNA was synthesized using M-MLV reverse transcriptase (TransGen Biotech, Beijing, China). The transcription levels of the metal transporter related genes were

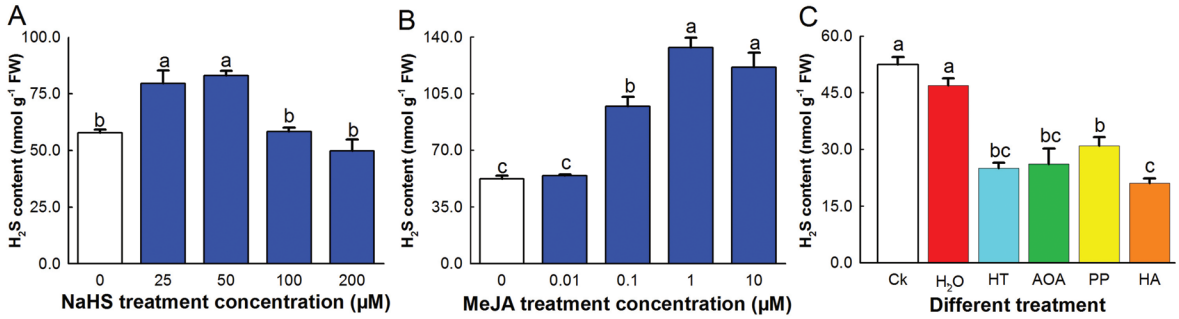

Figure 1. H_2S changes in different treated seedlings. (A) NaHS concentrations (25, 50, 100 and 200 μM), (B) MeJA concentrations (0.0.1, 0.1., 1 and 10 μM) and (C) H_2S inhibitors or scavengers (1000 μM) used in 12 h treatments. Data represents the mean ± SE of 30 seedlings with at least three independent repeats, and the different letters indicate the significant differences at $p < 0.0.5$.

Table 1. The specific primers used for real-time quantitative PCR

Gene	Gene notation	Primer sequence		Length/(bp)
ACTIN	Millet_GLEAN_10003390	F: GGTATGGAGTCGCCTGGAATCC	R: GCGGTCAGCAATACCAGGGAAC	110
NRAMP1	Millet_GLEAN_10005347	F: CCCTGGATACGGAATCTTGT	R: CCCCATCTTTGTTTTGCTAC	183
NRAMP6	Millet_GLEAN_10031864	F: TGAAGAAATGGCTGAGGAAC	R: GCAACCACGAGAACACGATG	231
MTP1	Millet_GLEAN_10025755	F: GCACTCCCCGTGAGATTGA	R: AGCTTCCCTTGCGATTGTT	146
MTP12	Millet_GLEAN_10002633	F: TTCTGCTGAAATTCTGTTGC	R: GTGAAAAGTGCCTACGATGT	154
CAX2	Millet_GLEAN_10017989	F: CTTGGCTGTGCTTTCTTTGC	R: ACATACTGCGGTGGCTCTTT	259
HMA3	Millet_GLEAN_10013711	F: TCTCGTCGGGCTATTTTCA	R: CTGCTCCTGTGCGTGCTT	139
ZIP4	Millet_GLEAN_10013362	F: GTCCGCTCTGTCGTCGTGT	R: GCCATTAGAACCGCTGAAA	209

detected using RT-qPCR performed on a CFX96TM C1000 Thermal Cycler System (Bio-Rad, Hercules, CA, USA). RT-qPCR was conducted in a 20-μL reaction containing 2 μL of diluted cDNA, 6.4. μL of distilled water, 0.8. μL of each primer and 10 μL of SYBR Green Real-time Master Mix (Toyabo, Japan). The primers for q-PCR are listed in Table 1. Annotation was performed using the foxtail millet database (http://foxtailmillet.genomics.org.cn/page/species/index.jsp).

3.6. Analysis of Cd accumulation

Determination of the Cd concentration was performed according to a previously described method (22). Briefly, the leaf and root samples were deactivated for 30 min at 100°C and then dried for 48 h at 80°C, weighed, ground into a fine powder, digested with HNO_3 and $HClO_4$ (3:1, v/v), and dissolved in deionized water. The Cd concentrations were determined using an atomic absorption spectrophotometer (AA240 VARIAN, Palo Alto, CA, USA).

3.7. Statistical analyses

All data are shown as the mean ± standard error (M±SE). Statistical analyses were performed using SPSS 17.0. software (IBM SPSS, Chicago, IL, USA). The statistical significance between the control and other treatment groups was determined by one-way analysis of variance, and different letters indicate significant differences at $p < 0.0.5$ according to Duncan's test.

4. RESULTS

4.1. Changes in H_2S content under different treatments

In this study, the effects of gradient concentrations of NaHS (25, 50, 100 and 200 μM) or MeJA (0.0.1, 0.1., 1 and 10 μM) and various H_2S modulators (HT, AOA, PP and HA at 1000 μM) on the endogenous H_2S concentrations were analyzed in seedlings. The results indicated that 50 μM NaHS significantly increased H_2S accumulation, while 100 or 200 μM NaHS had no effect (Figure 1A). MeJA (0.1., 1 and 10 μM) also substantially increased the endogenous H_2S concentrations (Figure 1B). Thus, 50 μM and 1 μM were selected as the effective concentrations for NaHS and MeJA, respectively. Meanwhile, the data indicated that HT, AOA, PP and HA treatment could significantly decrease the endogenous H_2S concentration compared to that of untreated seedlings (Figure 1 C). We chose 1000 μM HA as the treatment based on the change in endogenous H_2S.

4.2. Effects of MeJA and NaHS on plant growth

To investigate the relevance of MeJA and H_2S on seedling growth, the effects on shoot height and

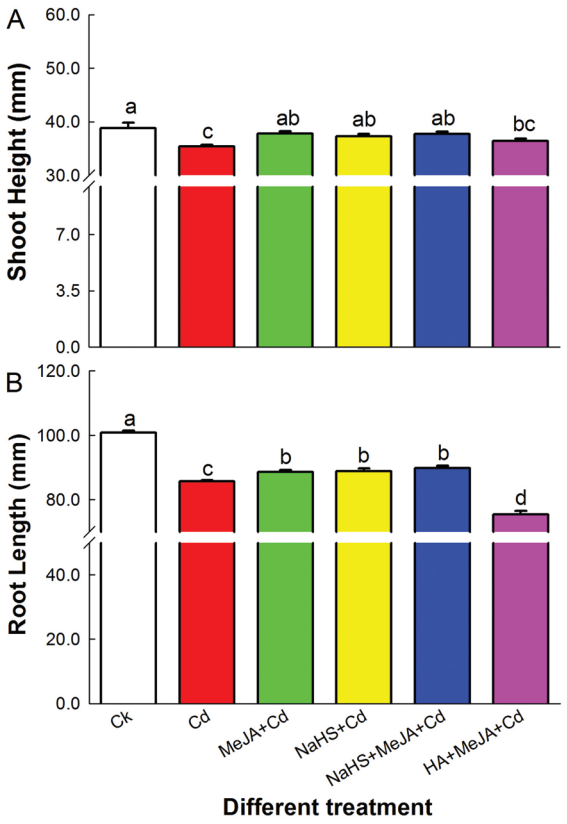

Figure 2. Effects of different treatments on the growth of the foxtail millet seedlings. (A) Shoot heights and (B) root lengths of the seedlings that underwent different treatments. Seedlings were treated with NaHS or HA for 12 h and then with MeJA+Cd or Cd for 48 h. Data represents the mean ± SE of 30 seedlings with at least three independent repeats, and the different letters on the columns indicate the significant difference at $p < 0.0.5$. Ck, untreated control; Cd, 200 μM $CdCl_2$ treatment; MeJA+Cd, 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; NaHS+Cd, 50 μM NaHS pretreatment followed by 200 μM $CdCl_2$ treatment; NaHS+MeJA+Cd, 50 μM NaHS pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; HA+MeJA+Cd, 1000 μM HA pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment.

root length were measured under different treatments (Figure 2). Compared to the control, treating with 200 μM Cd resulted in 6.8.% and 15% decreases in shoot height and root length, respectively. In the shoots and roots, both single and combined with MeJA and NaHS treatment significantly increased the shoot height and root length compared to Cd treatment. However, the root length was shorter than the MeJA+Cd treatment in the presence of HA, while the shoot height had no significant difference (Figure 2, A and B).

4.3. Histochemical staining

The effects of NaHS and MeJA on the alleviation of Cd-induced oxidative damage were examined by histochemical staining. DAB and NBT staining indicated H_2O_2 accumulation and O_2^- production in root, respectively. ROS accumulated in the root tips of Cd-treated seedlings compared to the control group, and H_2S and MeJA markedly decreased the ROS content under Cd stress (Figure 3, A and B). Roots treated with Cd alone were stained extensively with Schiff's reagent (Figure 3 C), whereas the NaHS+Cd or MeJA+Cd treatment led to slightly reduced staining depths. As shown in Figure 3 A, B and C, the staining of H_2O_2, O_2^- and lipid peroxidation was deeper than the MeJA+Cd treatment in the presence of HA. All of the results were consistent with the changes in MDA formation (Figure 3 D), a biomarker of lipid peroxidation for oxidative stress (28).

4.4. Effects of MeJA and H_2S on Cd accumulation

When seedlings were treated with 200 μM Cd treatment for 48 h, 79.1.9 mg g^{-1} (dry weight) Cd accumulated in the roots and the Cd content in the shoot was 44.1.6 mg g^{-1} (dry weight), which was dramatically higher than in the control group ($p < 0.0.1$) (Figure 4). Compared to the Cd-treated seedlings, the Cd content in the roots and shoots was significantly reduced in the MeJA+Cd and NaHS+Cd treatments ($p < 0.0.5$). Furthermore, the NaHS pretreatment decreased the Cd content by 34.3.% and 24.1.% in the roots and shoots, respectively, compared to the MeJA+Cd treatment alone. However, in the presence of HA, the reduction of the Cd content in the roots was partly reduced compared to the NaHS+MeJA+Cd-treated group (Figure 4 A), and there was no effect on the shoots (Figure 4 B).

4.5. Transcriptional expression changes of genes involved in metal uptake

To better determine molecular mechanism behind the altered Cd accumulation in the roots that underwent different treatments, the mRNA transcription levels of the genes involved in metal uptake were analyzed (Figure 5). Among the NRAMP-encoding genes in foxtail millet, we detected the mRNA transcription levels of *SiNRAMP1* and *SiNRAMP6* in the roots and found that their mRNA expression levels were significantly up-regulated by the Cd treatment ($p < 0.0.5$). Furthermore, compared to the Cd treatment alone, the MeJA+Cd, NaHS+Cd and NaHS+MeJA+Cd treatments down-regulated the expression of *SiNRAMP1* and *SiNRAMP6*. However, an HA pretreatment attenuated the decreased mRNA expression of those genes in the MeJA+Cd treatment group (Figure 5, A and B).

4.6. Transcriptional expression changes of genes involved in metal homeostasis

To understand the changes of intracellular Cd homeostasis in roots, the mRNA expression levels of the genes involved in metal homeostasis were further analyzed under different treatments (Figure 6). Five genes, including *MTP1* and *MTP12* encoding metal tolerance proteins, *CAX2* encoding a cation exchanger, *HMA3* encoding a heavy metal ATPase 3 and *ZIP4* encoding an iron-regulated transporter protein, were selected. Compared to the control group, the expression

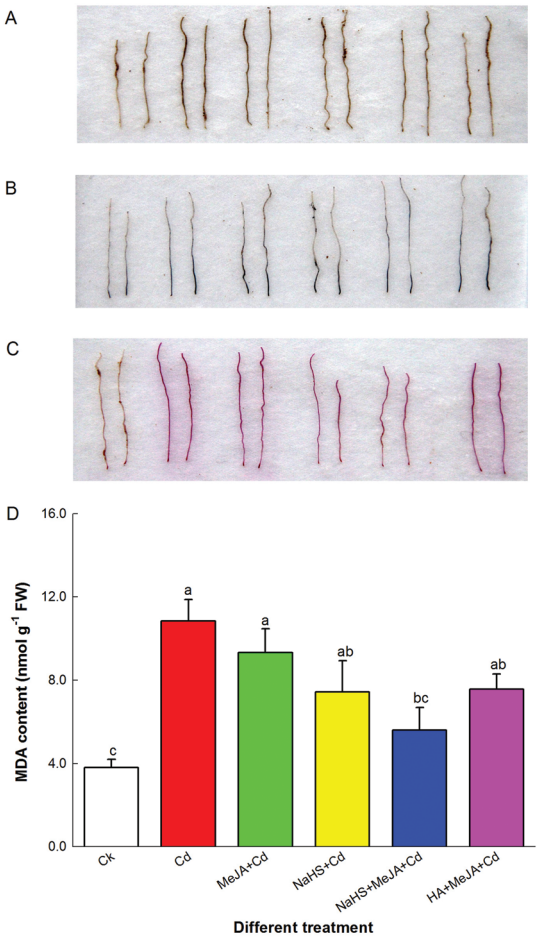

Figure 3. Effects of different treatments on several indexes of oxidative damage to the foxtail millet roots. Histochemical staining of (A) H_2O_2, (B) O_2^-, (C) lipid peroxidation and (D) MDA content in the roots that underwent different treatments. Seedlings were treated with NaHS or HA for 12 h and then with MeJA+Cd or Cd for 48 h. Data represents the mean ± SE of 30 seedlings with at least three independent repeats, and the different letters on the columns indicate the significant differences at $p < 0.0.5$. Ck, untreated control; Cd, 200 μM $CdCl_2$ treatment; MeJA+Cd, 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; NaHS+Cd, 50 μM NaHS pretreatment followed by 200 μM $CdCl_2$ treatment; NaHS+MeJA+Cd, 50 μM NaHS pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; HA+MeJA+Cd, 1000 μM HA pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment.

levels of these genes were increased to varying degrees after Cd exposure for 12 h (Figure 6 A - E). However, both MeJA+Cd and NaHS+Cd treatments up-regulated the *CAX2*, *MTP1* and *MTP12* expression levels; down-regulated *HMA3* expression; and had no effect on *ZIP4* compared to the Cd treatment (Figure 6). Compared to the MeJA+Cd treatment, the NaHS pretreatment did not affect the *MTP12* expression, but induced 1.2.-, 2.4.- and 4.6.-fold increases in the expression levels of *MTP1*, *CAX2* and *ZIP4*, respectively, and reduced *HMA3* expression 2.7.-fold. However, in the presence of HA pretreatment, these effects were negatively correlated.

5. DISCUSSION

Several previous studies have found that the addition of exogenous MeJA was associated with Cd tolerance in plants (18-20). Seedling growth was hampered by Cd-induced oxidative injury, including the rise of H_2O_2 or O_2^- levels and increased lipid peroxidation. In the present study, seedlings that underwent MeJA pretreatment had significantly decreased Cd levels (Figure 4), and similar findings were demonstrated for *Kandelia obovata* and *Capsicum frutescens* (18, 19). Interestingly, the addition of MeJA partially reversed the inhibitory effects of Cd on root and shoot growth (Figure 2). However, MeJA (50 μM) inhibited root growth of *Phaseolus coccineus*, *Allium cepa* and *Zea mays*, and this inhibition lasted throughout the experiment; however, there was no effect on *Allium cepa* leaf growth (29). Therefore, MeJA might have different regulatory mechanisms involved in Cd^{2+} inhibitory actions for root and leaf growth, which could be due to the diversity of plant species and the exogenous MeJA concentration.

Heavy metal stresses are the major limiting factors for crop productivity and grain quality. MeJA exhibited protective effects in plants against Cd stress (20), but the signal transmission mechanism for MeJA-reduced Cd toxicity was unclear in the different plant species. MeJA significantly reduced the Cd content in roots and shoots and that H_2S enhanced the role of MeJA, further reducing its accumulation (Figure 4), while the role of MeJA-reduction in roots was weakened by HA pretreatment. H_2S signaling participated in MeJA-induced Cd tolerance in foxtail millet, and H_2S acts as a downstream molecule of SA-transmitted signals to regulate Cd tolerance in *Arabidopsis* and the NO-activated H_2S response to Cd stress in bermudagrass (6, 7).

The reduction in Cd toxicity may be primarily due to a regulatory mechanism involved in metal homeostasis (uptake, transport and efflux) and metal detoxification. Recent reports showed that MeJA decreased the Cd uptake in rice and inhibited its translocation in *K. obovata* seedlings for antidotal actions (18, 30). MeJA weakened the up-regulated expression of *SiNRAMP1* and *SiNRAMP6*, which were markedly induced by Cd in roots, and H_2S affected the functionality of MeJA (Figure 5). Both AtNRAMP6 in *Arabidopsis* and OsNRAMP1 in rice accelerated Cd toxicity (31, 32). Accordingly, H_2S transmitted the effects of MeJA on the Cd-uptake reduction and NRAMPs are partly responsible for Cd uptake in the roots of this species (Figure 5). To cope with intracellular Cd, plants possess a strict metal homeostasis mechanism (21). Thus, the expression levels of other metal transporter genes, such as *MTP1*, *MTP12*, *CAX2*, *HMA3* and *ZIP4* in foxtail millet root were up-regulated by Cd, and MeJA via H_2S further enhanced the functions of MTPs and CAX2 in transporting metal ions into vacuoles for antidotal actions (Figure 6). H_2S

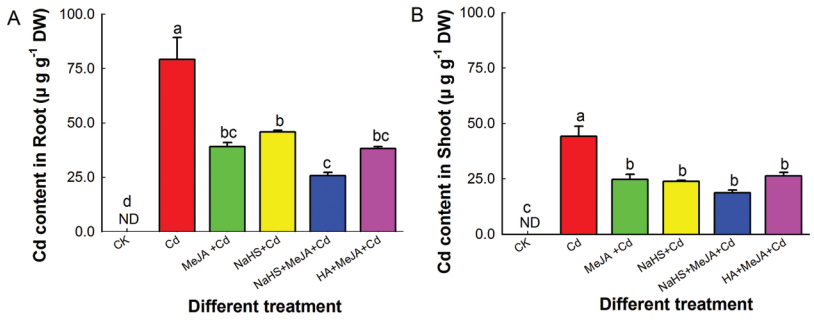

Figure 4. Effects of different treatments on the Cd content in the foxtail millet roots and shoots. Cd content in the (A) roots and (B) shoots of the seedlings that underwent different treatments. The seedlings were treated with NaHS or HA for 12 h and then with MeJA+Cd or Cd for 48 h. Data represents the mean ± SE of 30 seedlings with at least three independent repeats, and the different letters on the columns indicate the significant differences at $p < 0.0.5$. Ck, untreated control; Cd, 200 μM $CdCl_2$ treatment; MeJA+Cd, 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; NaHS+Cd, 50 μM NaHS pretreatment followed by 200 μM $CdCl_2$ treatment; NaHS+MeJA+Cd, 50 μM NaHS pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; HA+MeJA+Cd, 1000 μM HA pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment.

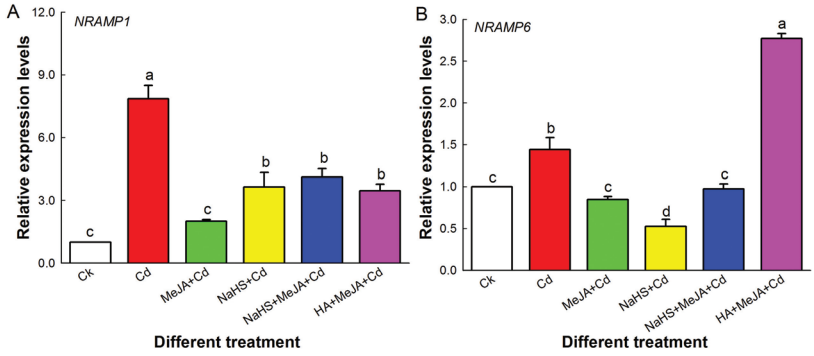

Figure 5. Expression levels of gene encoding metal uptake transporters in the roots that underwent different treatments. The seedlings were treated with NaHS or HA for 12 h and then with MeJA+Cd or Cd for 12 h. Data represents the mean ± SE of 30 seedlings with at least three independent repeats, and the different letters on the columns indicate the significant differences at $p < 0.0.5$. Ck, untreated control; Cd, 200 μM $CdCl_2$ treatment; MeJA+Cd, 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; NaHS+Cd, 50 μM NaHS pretreatment followed by 200 μM $CdCl_2$ treatment; NaHS+MeJA+Cd, 50 μM NaHS pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; HA+MeJA+Cd, 1000 μM HA pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment.

can also regulate Zn homeostasis involving the *ZRT*, *IRT*, *NRAMP*, *MTP* and *HMA4* genes in *Solanum nigrum* (33). Thus, H_2S might act as an important factor in the signaling network mediating the plant response to heavy metal stress. Due to the metal efflux direction in the roots, which depends on the metal accumulation phenotype of the plant, the foxtail millet system can be considered to be a novel research model that involves some efflux proteins such as PCR1, PCR2, PDR8 and HMA4 (21).

In addition to controlling cellular metals, plant cells possess a comprehensive metal detoxification system. Numerous molecules, such as glutathione (GSH), metallothioneins (MTs), phytochelatins (PCs) and amino acids, participate in Cd detoxification (21, 34). Exogenous H_2S applications usually affect intracellular responses, which increases the GSH levels, alters enzyme activities and regulates H_2O_2 metabolism (4). Meanwhile, MeJA also activates plant defense mechanisms, such as antioxidative capacity, AsA-GSH cycle and ROS metabolism, in response to Cd stresses (17-20). The relationship between H_2S and MeJA was due to their similar functions, which were involved in the induction of the Cd detoxification system. Other reports showed that H_2S could mediate signal transduction, along with other molecules such as NO, abscisic acid, gibberellic acid, H_2O_2 and SA (6-8, 13, 14), in response to many stresses.

Based on the aforementioned findings, we suggested that an interplay between MeJA and H_2S might exist during Cd stress. H_2S is a free signal element that is generated via either non-enzymatic or enzymatic mechanisms and performs its functions across cellular membranes. In the current study, treatment with 1 μM MeJA not only significantly increased endogenous H_2S content in seedlings (Figure 1) but also dramatically caused H_2S accumulation under Cd stress. Adversely, MeJA-induced Cd tolerance was weakened by the

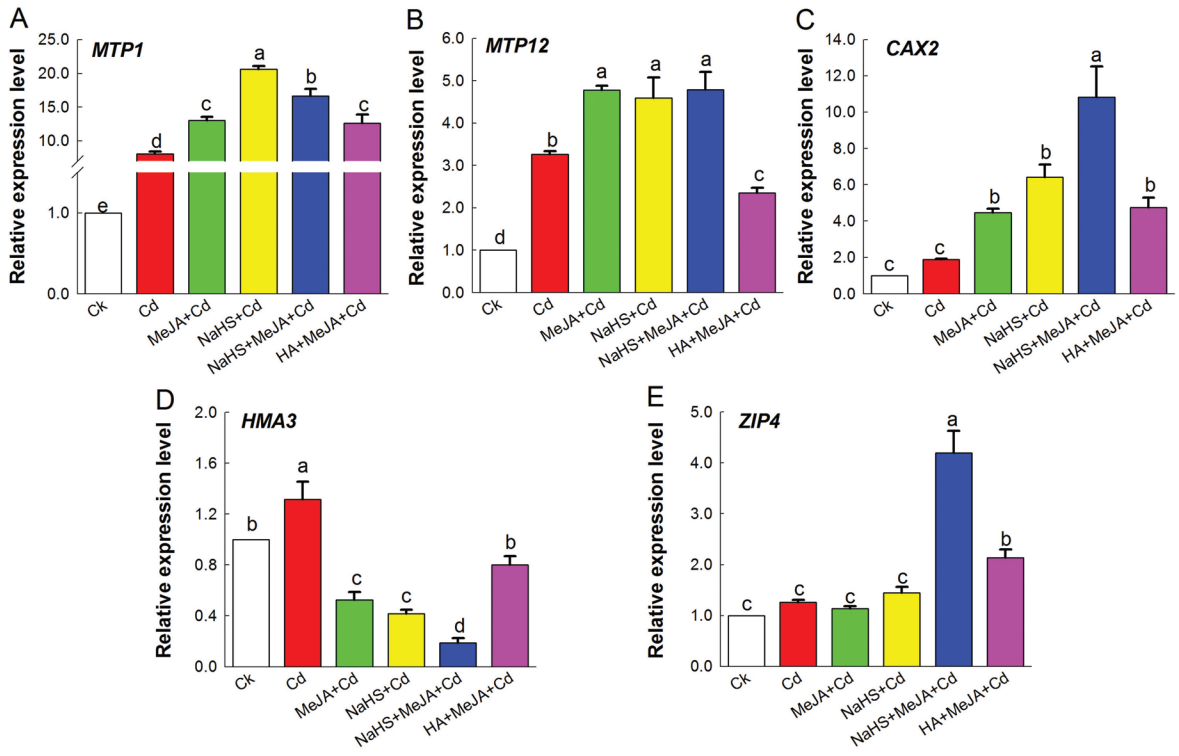

Figure 6. Expression levels of gene encoding metal transport proteins in the roots that underwent different treatments. The seedlings were treated with NaHS or HA for 12 h and then MeJA+Cd or Cd for 12 h. Data represents the mean ± SE of 30 seedlings with at least three independent repeats, and the different letters on the columns indicate the significant differences at $p < 0.0.5$. Ck, untreated control; Cd, 200 μM $CdCl_2$ treatment; MeJA+Cd, 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; NaHS+Cd, 50 μM NaHS pretreatment followed by 200 μM $CdCl_2$ treatment; NaHS+MeJA+Cd, 50 μM NaHS pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment; HA+MeJA+Cd, 1000 μM HA pretreatment followed by 1 μM MeJA and 200 μM $CdCl_2$ co-treatment.

HA pretreatment (Figures 2, 3, and 4). Thus, all of the data indicated that H_2S participated in the alleviation mechanism by which MeJA improved Cd tolerance in foxtail millet. Further studies are needed to investigate the regulatory mechanisms of endogenous JA and endogenous H_2S.

6. ACKNOWLEDGMENT

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Abbreviations: Cd, Cadmium; $CdCl_2$, cadmium chloride; H_2S, Hydrogen sulfide; MDA, Malondialdehyde; NaHS, Sodium hydrosulfide; AOA, Aminooxyacetic acid; HA, Hydroxylamine, PP, Potassium pyruvate; HT, Hypotaurine; PM, plasma membrane; ROS, Reactive oxygen species; H_2O_2, Hydrogen peroxide; O_2^-, Superoxide anion; JA, Jasmonic acid; SA, Salicylic acid; MeJA, Methyl jasmonate; ABA, Abscisic acid; NO, Nitric oxide; GSH, glutathione; MTs, metallothioneins; PCs, phytochelatins; ZIP, ZRT/IRT-Like proteins; Nramp, Natural resistance associated macro-phage protein; CAX, Cation/proton exchanger; HMA, Heavy metal ATPases; MTP, Metal tolerance protein.

Key Words: Hydrogen Sulfide, Methyl Jasmonate, Cadmium Damage, Foxtail Millet

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