Plant Physiology and Biochemistry 120 (2017) 112–119

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Hydrogen sulfide alleviates the cold stress through MPK4 in *Arabidopsis thaliana*

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ARTICLE INFO

Article history:
Received 13 September 2017
Received in revised form
29 September 2017
Accepted 30 September 2017
Available online 3 October 2017

Keywords:
Hydrogen sulfide
MPK4
Cold stress
Stomata

ABSTRACT

Hydrogen sulfide (H_2S) is a gaseous signaling molecule that mediates physiological processes in animals and plants. In this study, we investigated the relationship of H_2S and mitogen activated protein kinase (MAPK) under cold stress in Arabidopsis. H_2S up-regulated MAPK expression levels and was involved in the cold stress-related upregulation of MAPK genes expression. We then chose MPK4 whose expression level was influenced the most by H_2S as a target and found that H_2S's ability to alleviate cold stress required MPK4. Both H_2S and MPK4 regulated the expression levels of the cold response genes inducer of CBF expression 1 (*ICE1*), C-repeat-binding factors (*CBF3*), cold responsive 15A (*COR15A*) and cold responsive 15B (*COR15B*). H_2S inhibited the opening of stomata under cold stress, which required the participation of MPK4. In conclusion, MPK4 is a downstream component of H_2S-related cold-stress resistance, and H_2S and MPK4 both regulated the cold response genes and stomatal movement to response the cold stress.

1. Introduction

Hydrogen sulfide (H_2S) was named as the third gasotransmitter after NO and CO (Wang, 2014). H_2S was first studied in animals, but in recent years has been studied in plants. H_2S can be synthesized endogenously by multiple enzymes in plants. Desulfhydrase 1 (DES1) and L-cysteine desulfhydrase (LCD), that catalyze L-cysteine (L-cys) to H_2S, pyruvate and NH_3 (Álvarez et al., 2010; Papenbrock et al., 2007), are well-studied enzymes and are primarily responsible for H_2S generation because L-cys is the widespread form of cys in plants. H_2S participates in various physiological processes in plants, such as plant development, and biotic and abiotic (drought, cold and heavy metal) stress resistance through crosstalk with hormones, reactive oxygen species and some other molecular signals (Jin and Pei, 2015; Qiao et al., 2015; Cheng et al., 2013; Scuffi et al., 2014). Recently, the post-transcriptional molecular mechanism of H_2S was revealed. H_2S can regulate protein activity through the S-sulfhydrylation of cys sites in both mammals and plants (Mustafa et al., 2009; Aroca et al., 2015). In mammals, H_2S regulates many physiological processes through mitogen activated protein kinase (MAPK) (Li et al., 2011), but the relationship between H_2S and MAPK in plants has not yet been reported.

MAPK is an essential and conserved component of signal transduction pathways in eukaryotes and plays important roles in various signal responses (Šamajová et al., 2013; Smékalová et al., 2014). MAPK is composed of three cascades: MAPKKK (MAP3K or MEKK), MAPKK (MAP2K or MEK or MKK) and MAPK (MPK). When growth, stress or hormone signals occur, the MAPK pathway is activated in the plant. Firstly, MEKK will be activated through phosphorylation, then MEK will be phosphorylated by the activated MEKK, which will subsequently phosphorylate MPK. The activated MPK then regulates the target, such as transcription factors and protein kinases, resulting in the signal's response. Thus, MAPK participates in the processes of growth differentiation, hormonal responses, and biotic and abiotic stress resistance (Rodriguez et al., 2010). There are 60 MEKK, 10 MEK and 20 MPK proteins in Arabidopsis (Ichimura et al., 2002). To study the relationship between H_2S and MAPK in abiotic stress responses, we chose six representative members (MEKK1, MEK1, MEK2, MPK3, MPK4 and MPK6) that participate in responses to abiotic stresses, such as drought, heavy metal exposure and cold, in Arabidopsis (Furuya et al., 2013; Ichimura et al., 2000; Teige et al., 2004; Matsuoka et al., 2002;

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https://doi.org/10.1016/j.plaphy.2017.09.028
0981-9428/

Abbreviations

H_2S	Hydrogen sulfide
DES1	Desulfhydrase 1
LCD	L-cysteine desulfhydrase
Cys	Cysteine
MAPK	Mitogen activated protein kinase
MS	Murashige and Skoog
RT–PCR	Reverse transcription–PCR
qRT–PCR	quantitative real-time–PCR

Mizoguchi et al., 1996).

There are many functional overlaps between H_2S and MAPK, especially in biotic stress resistance. Furthermore, in mammals, H_2S mediates several processes through MAPK. Thus, we explored the relationship between H_2S and MAPK in abiotic stress resistance in Arabidopsis. Firstly, we detected the effects of H_2S on *MAPK* genes, and chose the gene that was highly influenced, *MPK4*. Because MPK4 is involved in cold-stress responses, we then studied the relationship between H_2S and MPK4 in cold-stress resistance using a root tip bending experiment, and the functions of H_2S and MPK4 on cold-response genes and stomatal movements under cold stress were also explored.

2. Materials and method

2.1. Plant materials and growth conditions

Arabidopsis thaliana of Columbia (Col) and Landsberg (Ler) ecotype were used. T-DNA insertion mutants of *LCD* (*lcd*; SALK-082099) and *DES1* (*des1*; SALK-205358C) were obtained from the Arabidopsis Biological Resource Center, and T-DNA insertion mutant of *mpk4* (Col) and homozygous *Ds* insertion mutant of *mpk4* (Ler) were provided by John Mundy of Copenhagen University generously. The growth conditions were described previously (Jin et al., 2011) with some modifications. Briefly, the seeds were soaked in distilled water and stored at 4 °C for 1 d. Then, the seeds were grown in the pots containing a soil:perlite:vermiculite (1:1:1 v/v) mixture, or the seeds were grown on 1/2 Murashige and Skoog (MS) medium in plates after being sterilizing with 75% ethyl alcohol and 6% NaClO. They were maintained at 23 °C, with 60% relative humidity, a 16 h/8 h (light/dark) photoperiod and 160 $\mu Emm^{-2}S^{-1}$ light illumination.

2.2. Genotyping of mutants

The DNA of seedlings was extracted for genotyping. The seeds of *des1* from ABRC is homozygous, we confirm that through genotyping, and the primers were des1-LP, des1-RP and LBb1.3 which was designed to detect T-DNA insert. The genotyping of *lcd* was described previously (Jin et al., 2013) and the primers were defined as P1, P2 and P3. The primers for *mpk4* (Col) were mpk4-col-RP, mpk4-col-LP and LBb1.3. The primers for *mpk4* (Ler) were mpk4-1-rew and DS1 which was designed to detect the *DS* insertion. The primers were listed in Table 1 (supplement. Table S1).

2.3. Total RNA extraction, reverse transcription (RT)–PCR and quantitative real-time (qRT)–PCR

Seedling samples of 0.03–0.05 g with 1 ml of RNAiso Plus (TaKaRa, Shiga, Japan) added were used for total RNA extraction following the manufacturer's instructions. Isolated RNA was used in RT–PCR, and cDNA was generated using EasyScript Reverse Transcriptase (TransGen Biotech, Beijing, China) and Ribonuclease Inhibitor (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Then, the cDNA was used as templates, and the target gene' expression levels were detected using qRT–PCR, which was described previously (Shen et al., 2013). The primers were listed in Table 1 (supplement. Table S1), and *UBQ* was used as an internal control.

2.4. Measurement of the endogenous H_2S content

The method used to measure the H_2S content was described previously (Lai et al., 2014). The only modification was that the seedlings were extracted in 50 mM phosphate buffer solution (pH 6.8) containing 0.1 mM EDTA and 0.2 mM ASA.

2.5. Root tip bending experiment

The seeds were grown on 1/2 MS plates and cultured vertically as described in Section 2.1 for 7 d. After the NaHS (H_2S donor) treatment, the seedlings were transferred to new 1/2 MS plates and then were placed back into normal conditions (23 °C) or into cold conditions (4 °C) vertically and inversely. Non-treated seedlings were used as controls. All of the parameters except the temperature were the same.

2.6. Stomatal aperture assay

The leaves cut from four-week-old seedlings were soaked into epidermal strip buffer (containing 10 mM MES and 50 mM KCl, pH 6.15) with and without NaHS and put under light. Then the leaves soaked in epidermal strip buffer were put in 4 °C or not under light. After undergoing cold stress or not, the abaxial epidermis was peeled from the leaves. The epidermal strip was immediately placed in epidermal strip buffer on a glass side and covered with a coverslip. The pore widths of 20–30 stomata from the epidermal strip were observed using an optical microscope (OLYMPUS, Tokyo, Japan: CH20BIMF200).

2.7. Statistical analysis

Data are presented as the means ± standard error (SE). Statistical analyses were performed using SPSS version 17.0 (SPSS, IBM, Chicago, IL, USA), and significance analysises were performed using a one-way analysis of variance followed by Duncan's test. ** represents highly significantly different ($P < 0.01$), * and different letters represent significantly different ($P < 0.05$), respectively. For every result, three independent experiments were conducted.

3. Results

3.1. H_2S upregulated the gene expression levels of MAPKs

Firstly, we examined whether *MAPK* gene expressions are influenced by NaHS. Endogenous H_2S content increased after NaHS fumigation (Fig. 1a). After 3 h of NaHS fumigation, the gene expression levels of *MEK2*, *MPK4* and *MPK6* started to increase and was maintained until 9 h, and gene expression levels of *MEKK1*, *MEK1* and *MPK3* also increased significantly after 9 h of NaHS fumigation (Fig. 1b).

We got homozygous mutants of H_2S-generated enzymes DES1 and LCD, *des1* and *lcd* (supplement Fig. S1), in which the *DES1* and *LCD* expressions decreased, and the H_2S content was lower than in the wild type (WT) (Fig. 1c–e). We also detected the expression

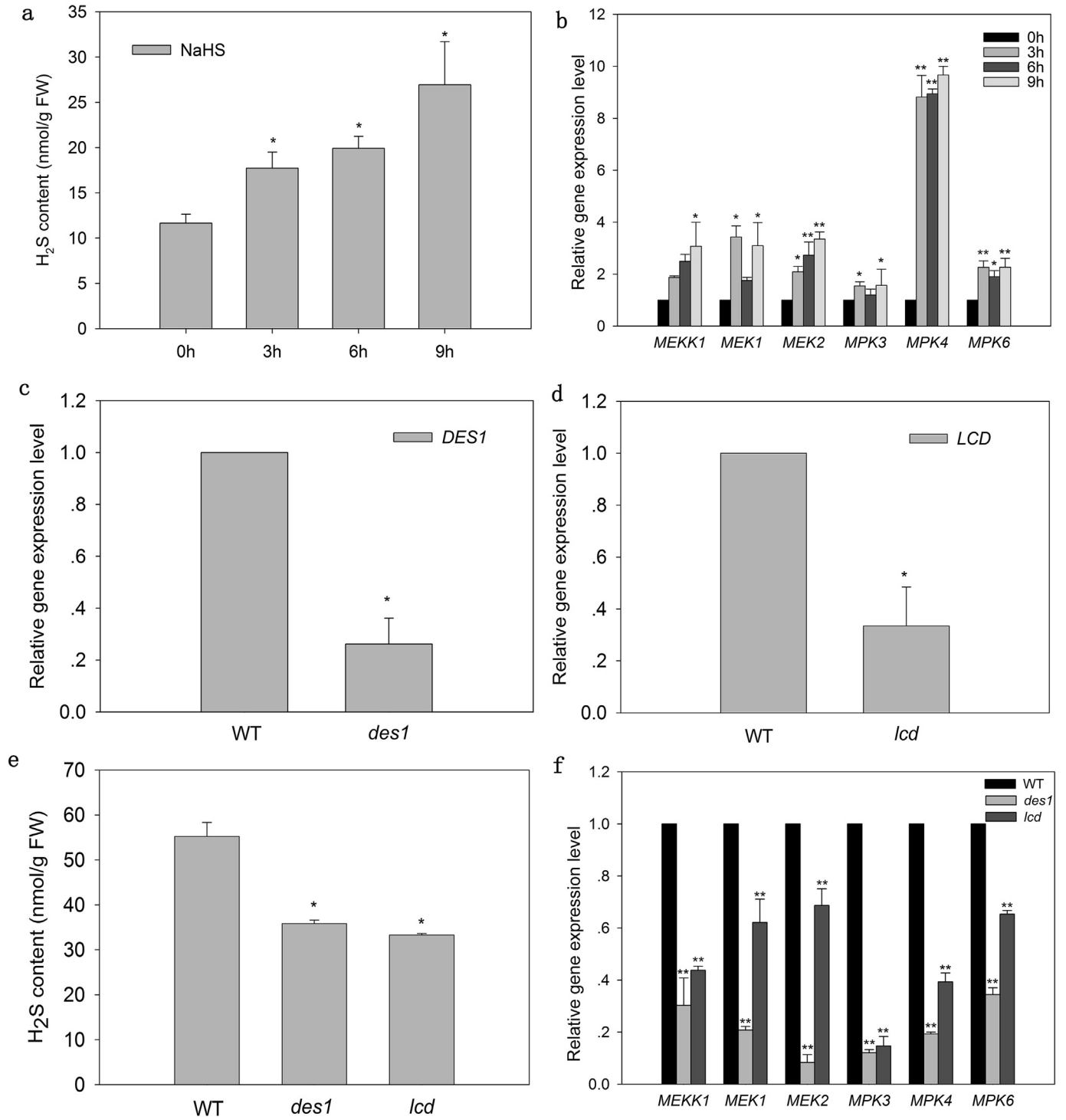

Fig. 1. Effect of H_2S on gene expression of *MAPKs* in Arabidopsis. a: H_2S content after NaHS fumigation in WT. b: gene expression level of *MAPK* after NaHS fumigation in WT. c-d The gene expression level of *DES1* in *des1* and *LCD* in *lcd*. e: H_2S content in WT, *des1* and *lcd*. f. gene expression level of MAPK in WT, *des1* and *lcd*. 4-week-old seedlings of WT, *des1* and *lcd* of Col ecotype Arabidopsis were used. Seedlings were fumigated by 50 μM NaHS for different time (0, 3, 6, 9 h). Date are Mean ± SE and at least three independent experiments were repeated.

levels of *MAPKs* in *des1* and *lcd*. In both of mutants, the gene expression levels of *MAPKs* decreased compared with in the WT (Fig. 1f).

3.2. Cold-stress induction of MAPK genes mediated by H_2S

MAPK plays a roles in cold-stress resistance, therefore, we studied whether H_2S is involved in this process. Cold stress could upregulate the gene expression levels of *MAPKs* in WT (Fig. 2a). However, in the *des1*, this effect of cold stress disappeared; moreover, the expression levels of *MEK1*, *MPK3* and *MPK6* decreased after the cold stress (Fig. 2b). In the *lcd*, the *MAPK* expression levels were not induced by cold stress, except *MEKK1*, which increased significantly less than in WT. The expression levels of *MPK3*, *MPK4*

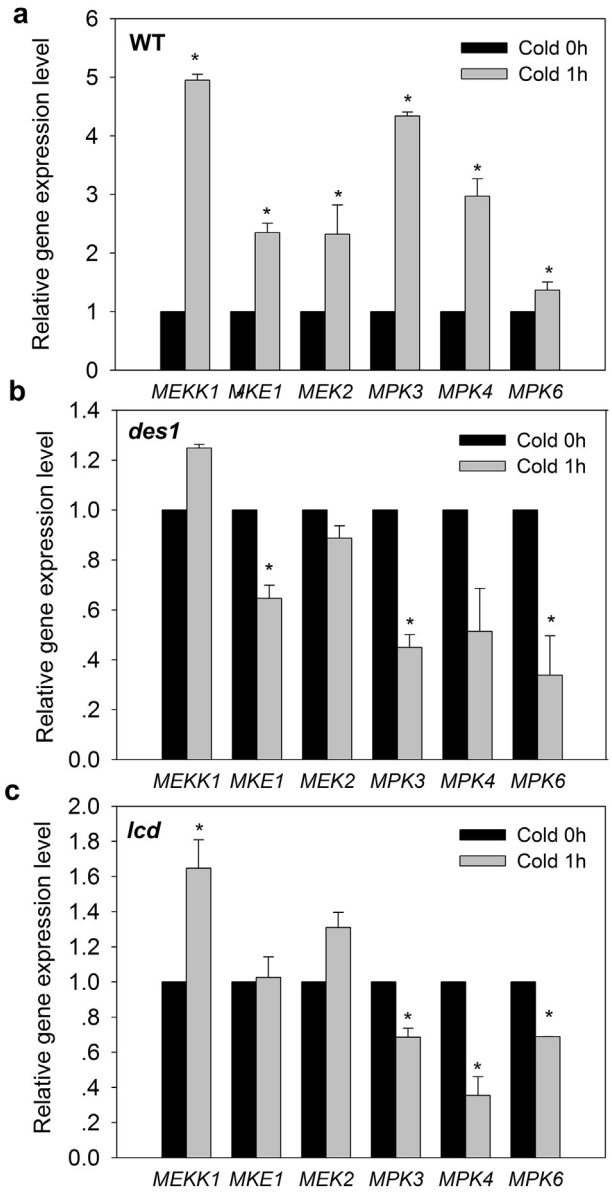

Fig. 2. The effect of cold stress on *MAPK* gene expression in WT (a), *des1* (b), *lcd* (c). 4-week-old seedlings of WT, *des1* and *lcd* of Col ecotype Arabidopsis were exposed to 4 °C for 1 h or not before extracting RNA. Date are Mean ± SE and at least three independent experiments were repeated.

and *MPK6* decreased after cold stress in the *lcd* (Fig. 2c).

3.3. H_2S alleviated cold stress through MPK4

Because *MPK4* was influenced the most by H_2S, and it plays an important role in cold-stress responses, we obtained the *mpk4* to explore the relationship between MPK4 and H_2S in cold-stress resistance. In the root tip bending experiment (Fig. 3a–b), the homozygous *mpk4* (Ler) was used (Supplement Fig. S2) and the hook length of the root was observed. The growth of the root was inhibited under cold stress in both WT and the *mpk4*. A NaHS pretreatment alleviated the inhibition of cold stress on root growth in the WT, while in *mpk4*, the pretreatment did not alleviate the inhibition under cold stress.

3.4. H_2S and MPK4 both regulate the expression levels of cold-response genes

Inducer of CBF expression 1 (*ICE1*), *C-repeat-binding factors* (*CBF3*), *cold responsive 15A* (*COR15A*) and *cold responsive 15B* (*COR15B*) are crucial cold-response gene that could increase cold-stress resistance. To study the mechanism of H_2S alleviating cold stress through MPK4, we studied the effects of MPK4 and H_2S on these four genes. The homozygous *mpk4* (col) was used (Supplement Fig. S3). In the *mpk4*, the expression levels of *ICE1*, *COR15A* and *COR15B* decreased significantly compared with in WT (Fig. 4a). In *des1* and *lcd*, *ICE1* and *CBF3* expression levels decreased. The gene expression levels of *COR15A* and *COR15B* did not change in *des1* and *lcd*, except that *COR15A*'s expression increased slightly in the *lcd* (Fig. 4b).

Because the functions of H_2S are more obvious under stress conditions, we continued to explore the effects of H_2S on these cold response genes under cold stress. In WT, the expression levels of *ICE1*, *CBF3*, *COR15A* and *COR15B* were induced after 2 h of cold stress. The increases in the four genes' expression levels were inhibited in *des1* and *lcd* under cold stress (Fig. 4c–f).

3.5. H_2S inhibits the stomatal opening in a MPK4-dependent manner under cold stress

MPK4 functions in stomatal closure, and stomatal movement is another important process in the response to the cold stress. Therefore, we studied the effects of H_2S and MPK4 on stomatal movement under cold stress, and *mpk4* (Ler) was used. In WT, the aperture of the stomata decreased after the NaHS treatment and cold stress compared with CK, while NaHS and cold co-treatment decreased the aperture more (Fig. 5a). In the *mpk4*, the stomatal aperture decreased after cold treatment but did not change after the NaHS treatment compared with in the CK. After the NaHS and cold co-treatment, the stomatal aperture was the same as those under cold treatment in the *mpk4* (Fig. 5b).

4. Discussion

In mammals, the relationship between H_2S and MAPK has been well studied, and H_2S is involved in numerous physiological processes through its regulation of MAPK activity (Xuan et al., 2012; Li et al., 2011; Papapetropoulos et al., 2009; Yang et al., 2004). In plants, study on the relationship between H_2S and MAPK has not yet been reported. Here, we provide a preliminary report on the relationship between H_2S and MAPK in Arabidopsis. Fig. 1 shows that H_2S could upregulate the gene expression levels of *MAPKs*, suggesting that H_2S has a close relationship with MAPK in plants. *MPK4* is the gene whose expression increased the most after the H_2S treatment, indicating that the physiological processes of H_2S regulation may be mediated by MPK4.

Cold stress is a common stress to plants. MAPK and H_2S are both involved in cold-stress resistance in plants. The MEKK1→MEK2→MPK4 cascade is a famous pathway that participates in the cold- and salt-stress responses of Arabidopsis (Teige et al., 2004). Additionally, reports have linked H_2S to plant cold resistance in Arabidopsis, bermudagrass and *Lamiophlomis rotata* (Shi et al. 2013, 2015a; Ma et al., 2015), in which the production of H_2S increased under cold treatments and pretreatments of NaHS decreased the damage caused by cold stress. In these studies, the preliminary relationship between H_2S and cold-stress resistance in plants was revealed, but the mechanism was still unexplored. Fig. 2 shows that cold stress upregulated the gene expression levels of the

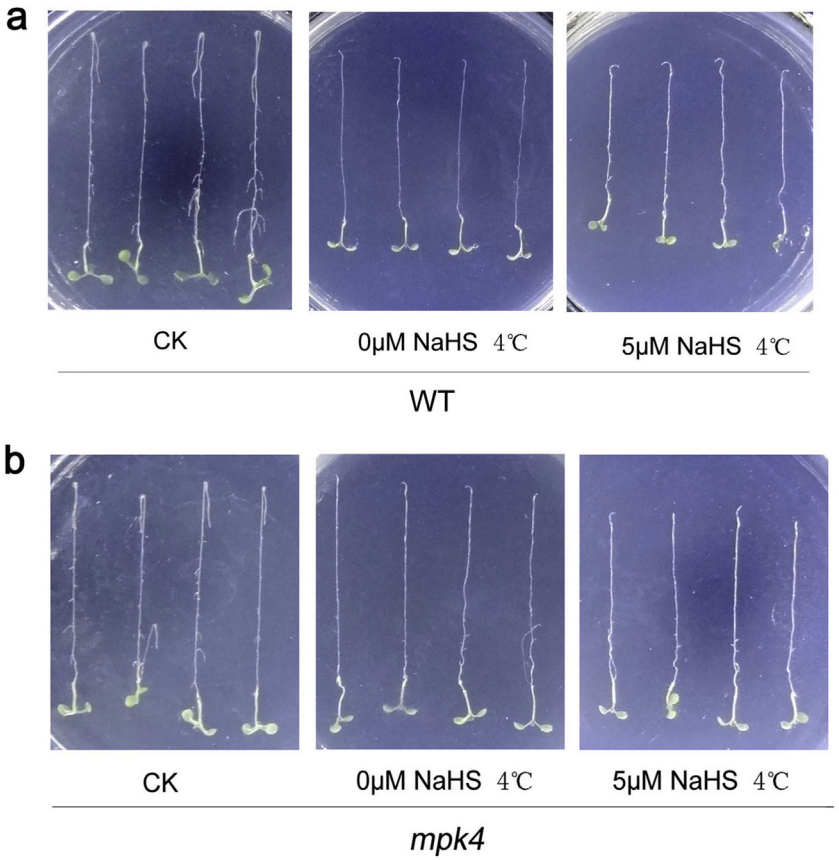

Fig. 3. The alleviation effect of H_2S in WT (a) and *mpk4* (b) under cold stress. 1-week-old seedlings of Ler ecotype Arabidopsis that were grown on the 1/2 MS medium were fumigated by 5 μM NaHS for 12 h or not before putting in 4 °C treatment inversely. CK was still grown in 23 °C inversely. The hook of root tip bending was observed 2 d later. At least three independent experiments were repeated.

*MAPK*s and that the process required the participation of H_2S. Fig. 3 shows that the alleviation of H_2S to cold stress was mediated by MPK4, indicating that H_2S is upstream of MPK4 in cold-stress responses.

The ICE1–CBF–COR transcriptional cascade is the best understood cold-stress response pathway. ICE1 is the transcription factor of *CBF3*, and CBF3 is the transcription factor of the *COR* genes. They are all induced by cold stress (Shi et al., 2015b). MEK2 up-regulates the gene expression of *CBF2* and *CBF3* in Arabidopsis (Teige et al., 2004). However, whether MPK4 regulates these genes is still not known. Fig. 4a shows that MPK4 up-regulated the gene expression of the cold response genes *ICE1*, *COR15A* and *COR15B*. However, the expression of *CBF3* was not influenced in the *mpk4*. It is reported that the gene expression level of *CBF3* decreased in the *lcd* (Shi et al., 2015a). Fig. 4b shows that the expression levels of *ICE1* and *CBF3* decreased in both *des1* and *lcd*, but the levels of *COR15A* and *COR15B* did not change, indicating that H_2S affects the expression levels of *ICE1* and *CBF3* but not those of *COR15A* and *COR15B*. The ICE1–CBF3–COR cascade is regulated at transcriptional, post-transcriptional and posttranslational levels. The stability of ICE1 is regulated by ubiquitination and sumoylation. The expression of *CBF3* is not only regulated by ICE1 but also by MYB15, ZAT12 and EIN3. There are two homologs of *CBF3*, *CBF1* and *CBF2*, which also regulate the gene expression levels of the COR genes (Shi et al., 2015b). We inferred that, owing to the complexity, not all four cold response genes' expression levels were affected in the *mpk4*, *des1* and *lcd*. Under cold stress, the ability of H_2S to regulate the four cold response genes became obvious (Fig. 4c–f). Thus, MPK4 and H_2S both regulate the expression levels of cold response genes. Based on Figs. 2–4, we hypothesized that the $H_2S \rightarrow$ MEKK1 $\rightarrow$ MEK2 $\rightarrow$ MPK4 $\rightarrow$ cold response genes cascade participates in the cold stress response process.

Numbers of MAPK members were reported to be involved in stomatal movement (Su et al., 2017; Li et al., 2017). *MPK4* is highly expressed in the guard cells of Arabidopsis (Petersen et al., 2000) and in the abaxial epidermis of tobacco in which it functions in stomatal closure (Gomi et al., 2005). This indicates that MPK4 is involved in stomatal movements in Arabidopsis. According to Fig. 5, H_2S could inhibit the stomatal opening at a normal temperature in the WT, while in the *mpk4* mutant this function was abolished, suggesting that MPK4 participated in the H_2S-related inhibition of stomatal opening. Stomatal closure under cold stress can protect the plant from injury caused by cold stress (Wilkinson et al., 2001). As Fig. 5 shows, cold stress inhibited stomatal opening in WT and *mpk4* mutants, and an NaHS treatment aggravated this inhibition in WT but not in the *mpk4* mutant. Thus, H_2S prevents stomatal opening under cold stress and this process requires MPK4.

Every molecule in an organism contains information regarding our survial on earth. H_2S, which is a prevalent gas, existed in the Archean eon of the earth (Olson and Straub, 2016), and MAPK, which is a conserved molecule in eukaryotes, may have interacted with each other during the origin of life to help living organisms to adapt the environment. Here, we show that H_2S can increase ability of cold stress resistance through MPK4 in Arabidopsis, resulting in a better adaptability to the environment.

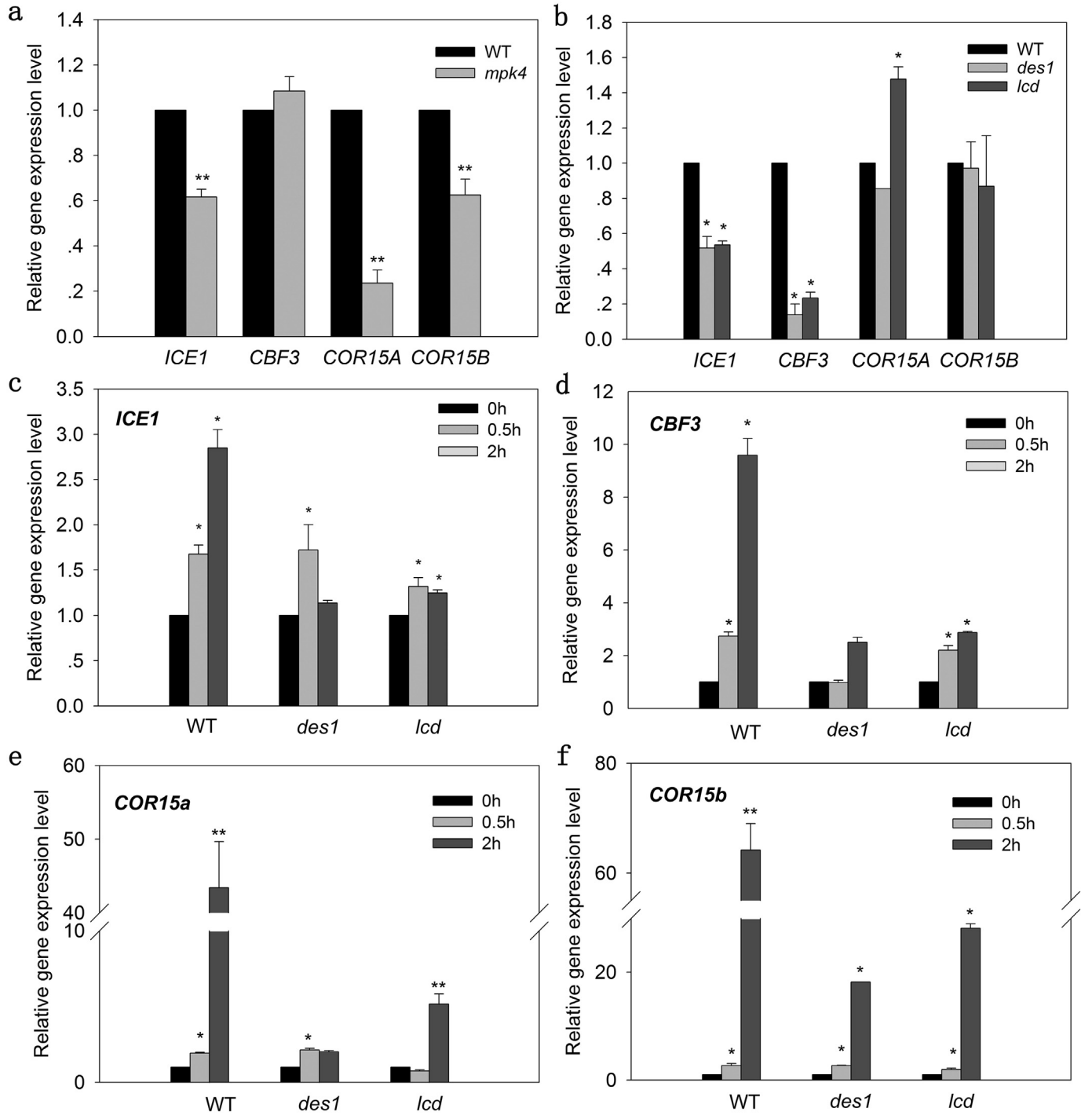

Fig. 4. The effect of H_2S and MPK4 on cold response genes in Arabidopsis. a: the gene expression level of cold response genes in WT and *mpk4*. b: the gene expression level of cold response genes in WT, *des1* and *lcd*. 4-week-old seedlings of WT, *mpk4*, *des1* and *lcd* of Col ecotype were used for extracting RNA. c–f: the gene expression level of cold response genes under cold stress in WT, *des1* and *lcd*. 4-week-old seedlings of WT, *des1* and *lcd* of Col ecotype were exposed to 4 °C for different time before extracting RNA. Date are Mean ± SE and at least three independent experiments were repeated.

Authors' contributions

Xinzhe Du, Yanxi Pei and Guangdong Yang designed the experiments and wrote the manuscript. Xinzhe Du performed the experiments. Xinzhe Du, Zhuping Jin and Danmei Liu analyzed the data. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [grant numbers 31672140, 31671605]; Shanxi Province Hundred Talents Program of 2007; Shanxi Province Science Foundation for Youths [grant numbers 2016021097];

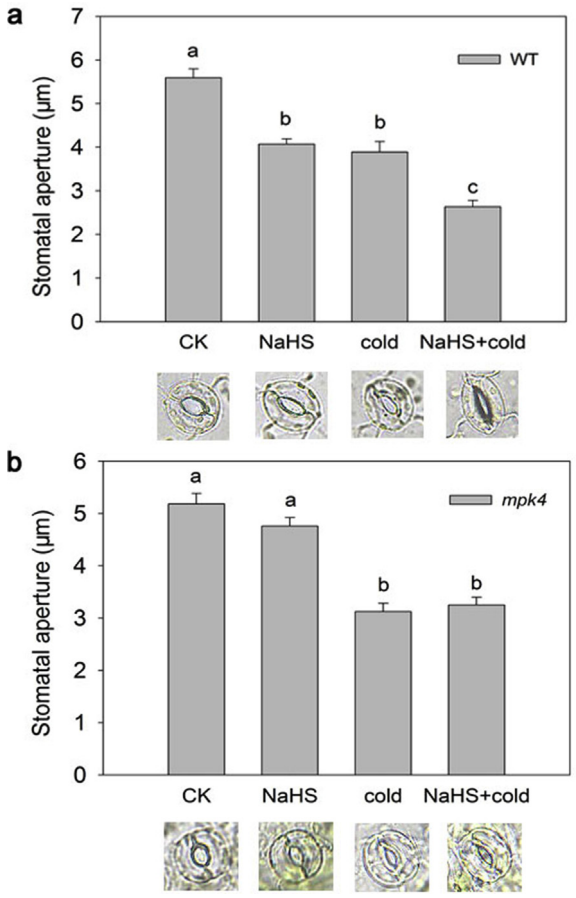

Fig. 5. The effect of H_2S on stomatal movement under cold stress in WT (a) and *mpk4* (b). The 4-week-old seedlings of WT and *mpk4* of Ler ecotype were used, and 20-30 stomatal aperture of the leaves were observed after 50 μM NaHS treatment for 30 min or not and 4 °C treatment for 1 h or not. Date are Mean ± SE and at least three independent experiments were repeated.

University Science and Technology Innovation Project [grant numbers 2016110]; and Shanxi Scholarship Council of China [grant numbers 2016-008]. We thank John Mundy of Copenhagen University for providing *mpk4* mutants seeds generously.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.plaphy.2017.09.028.

References

Álvarez, C., Calo, L., Romero, L.C., García, I., Gotor, C., 2010. An *O*-Acetylserine(thiol) lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in *Arabidopsis*. Plant Physiol. 152, 656–669. https://doi.org/10.1104/pp.109.147975.

Aroca, Á., Serna, A., Gotor, C., Romero, L.C., 2015. S-sulfhydration: a cysteine posttranslational modification in plant systems. Plant Physiol. 168, 334–342. https://doi.org/10.1104/pp.15.00009.

Cheng, W., Zhang, L., Jiao, C., Su, M., Yang, T., Zhou, L., Peng, R., Wang, R., Wang, C., 2013. Hydrogen sulfide alleviates hypoxia-induced root tip death in Pisum sativum. Plant Physiol. Biochem. 70, 278–286. https://doi.org/10.1016/j.plaphy.2013.05.042.

Furuya, T., Matsuoka, D., Nanmori, T., 2013. Phosphorylation of *Arabidopsis thaliana* MEKK1 via Ca^{2+} signaling as a part of the cold stress response. J. Plant. Res. 126, 833–840. https://doi.org/10.1007/s10265-013-0576-0.

Gomi, K., Ogawa, D., Katou, S., Kamada, H., Nakajima, N., Saji, H., Soyano, T., Sasabe, M., Machida, Y., Mitsuhara, I., Ohashi, Y., Seo, S., 2005. A mitogen-activated protein kinase NtMPK4 activated by SIPKK is required for jasmonic acid signaling and involved in ozone tolerance via stomatal movement in tobacco. Plant Cell Physiol. 46, 1902–1914. https://doi.org/10.1093/pcp/pci211.

Ichimura, K., Mizoguchi, T., Yoshida, R., Yuasa, T., Shinozak, K., 2000. Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6. Plant J. 24, 655–665. https://doi.org/10.1046/j.1365-313x.2000.00913.x.

Ichimura, K., Shinozaki, K., Tena, G., Sheen, J., Henry, Y., Champion, A., Kreis, M., Zhang, S., Hirt, H., Wilson, C., Heberle-Bors, E., Ellis, B.E., Morris, P.C., Innes, R.W., Ecker, J.R., Scheel, D., Klessig, D.F., Machida, Y., Mundy, J., Ohashi, Y., Walker, J.C., 2002. Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends Plant Sci. 7, 301–308. https://doi.org/10.1016/S1360-1385(02)02302-6.

Jin, Z., Pei, Y., 2015. Physiological implications of hydrogen sulfide in plants: pleasant exploration behind its unpleasant odour. Oxid. Med. Cell Longev. 2015, 397502. https://doi.org/10.1155/2015/397502.

Jin, Z., Shen, J., Qiao, Z., Yang, G., Wang, R., Pei, Y., 2011. Hydrogen sulfide improves drought resistance in *Arabidopsis thaliana*. Biochem. Biophys. Res. Commu 414, 481–486. https://doi.org/10.1016/j.bbrc.2011.09.090.

Jin, Z., Xue, S., Luo, Y., Tian, B., Fang, H., Li, H., Pei, Y., 2013. Hydrogen sulfide interacting with abscisic acid in stomatal regulation responses to drought stress in *Arabidopsis*. Plant Physiol. Biochem. 62, 41–46. https://doi.org/10.1016/j.plaphy.2012.10.017.

Lai, D., Mao, Y., Zhou, H., Li, F., Wu, M., Zhang, J., He, Z., Cui, W., Xie, Y., 2014. Endogenous hydrogen sulfide enhances salt tolerance by coupling the reestablishment of redox homeostasis and preventing salt-induced K^+ loss in seedlings of *Medicago sativa*. Plant Sci. 225, 117–129. https://doi.org/10.1016/j.plantsci.2014.06.006.

Li, K., Yang, F., Zhang, G., Song, S., Li, Y., Ren, D., Miao, Y., Song, C., 2017. AIK1, a mitogen-activated protein kinase, modulates abscisic acid responses through the MKK5-MPK6 kinase cascade. Plant Physiol. 173, 1391–1408. https://doi.org/10.1104/pp.16.01386.

Li, L., Rose, P., Moore, P.K., 2011. Hydrogen sulfide and cell signaling. Annu. Rev. Pharmacol. Toxicol. 51, 169–187. https://doi.org/10.1146/annurev-pharmtox-010510-100505.

Ma, L., Yang, L., Zhao, J., Wei, J., Kong, X., Wang, C., Zhang, X., Yang, Y., Hu, X., 2015. Comparative proteomic analysis reveals the role of hydrogen sulfide in the adaptation of the alpine plant *Lamiophlomis rotata* to altitude gradient in the Northern Tibetan Plateau. Planta 241, 887–906. https://doi.org/10.1007/s00425-014-2209-9.

Matsuoka, D., Nanmori, T., Sato, K., Fukami, Y., Kikkawa, U., Yasuda, T., 2002. Activation of AtMEK1, an *Arabidopsis* mitogen-activated protein kinase kinase, *in vitro* and *in vivo*: analysis of active mutants expressed in *E. coli* and generation of the active form in stress response in seedlings. Plant J. 29, 637–647. https://doi.org/10.1046/j.0960-7412.2001.01246.x.

Mizoguchi, T., Irie, K., Hirayama, T., Hayashida, N., Yamaguchi-Shinozaki, K., Matsumoto, K., Shinozaki, K., 1996. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A. 93, 765–769. https://doi.org/10.1073/pnas.93.2.765.

Mustafa, A.K., Gadalla, M.M., Sen, N., Kim, S., Mu, W., Gazi, S.K., Barrow, R.K., Yang, G., Wang, R., Snyder, S.H., 2009. H2S signals through protein S-sulfhydration. Sci. Signal 2. https://doi.org/10.1126/scisignal.2000464 ra72-ra72.

Olson, K.R., Straub, K.D., 2016. The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling. Physiology 31, 60–72. https://doi.org/10.1152/physiol.00024.2015.

Papapetropoulos, A., Pyriochou, A., Altaany, Z., Yang, G., Marazioti, A., Zhou, Z., Jeschke, M.G., Branski, L.K., Herndon, D.N., Wang, R., Szabo, C., 2009. Hydrogen sulfide is an endogenous stimulator of angiogenesis. PNAS 106, 21972–21977. https://doi.org/10.1073/pnas.0908047106.

Papenbrock, J., Riemenschneider, A., Kamp, A., Schulz-Vogt, H.N., Schmidt, A., 2007. Characterization of cysteine-degrading and H_2S-releasing enzymes of higher plants - from the field to the test tube and back. Plant Biol. 9, 582–588. https://doi.org/10.1055/s-2007-965424.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H.B., Lacy, M., Austin, M.J., Parker, J.E., Sharma, S.B., Klessig, D.F., Martienssen, R., Mattsson, O., Jensen, A.B., Mundy, J., 2000. *Arabidopsis* MAP kinase 4 negatively regulates systemic acquired resistance. Cell 103, 1111–1120. https://doi.org/10.1016/S0092-8674(00)00213-0.

Qiao, Z., Jing, T., Jin, Z., Liang, Y., Zhang, L., Liu, Z., Liu, D., Pei, Y., 2015. CDPKs enhance cd tolerance through intensifying H_2S signal in *Arabidopsis thaliana*. Plant Soil 398, 99–110. https://doi.org/10.1007/s11104-015-2643-x.

Rodriguez, M.C.S., Petersen, M., Mundy, J., 2010. Mitogen-activated protein kinase signaling in plants. Annu. Rev. Plant Bio. 61, 621–649. https://doi.org/10.1146/annurev-arplant-042809-112252.

Šamajová, O., Plíhal, O., Al-Yousif, M., Hirt, H., Šamaj, J., 2013. Improvement of stress tolerance in plants by genetic manipulation of mitogen-activated protein kinases. Biotechnol. Adva 31, 118–128. https://doi.org/10.1016/j.biotechadv.2011.12.002.

Scuffi, D., Álvarez, C., Laspina, N., Gotor, C., Lamattina, L., García-Mata, C., 2014. Hydrogen sulfide generated by L-cysteine desulfhydrase acts upstream of nitric oxide to modulate abscisic acid-dependent stomatal closure. Plant Physiol. 166, 2065–2076. https://doi.org/10.1104/pp.114.245373.

Shen, J., Xing, T., Yuan, H., Liu, Z., Jin, Z., Zhang, L., Pei, Y., 2013. Hydrogen sulfide improves drought tolerance in *Arabidopsis thaliana* by microRNA expressions. PLoS One 8, e77047. https://doi.org/10.1371/journal.pone.0077047.

Shi, H., Ye, T., Chan, Z., 2013. Exogenous application of hydrogen sulfide donor

sodium hydrosulfide enhanced multiple abiotic stress tolerance in bermudagrass (*Cynodon dactylon* (*L*). *Pers.*). Plant Physiol. Biochem. 71, 226–234. https://doi.org/10.1016/j.plaphy.2013.07.021.

Shi, H., Ye, T., Han, N., Bian, H., Liu, X., Chan, Z., 2015a. Hydrogen sulfide regulates abiotic stress tolerance and biotic stress resistance *in Arabidopsis*. J. Integr. Plant Biol. 57, 628–640. https://doi.org/10.1111/jipb.12302.

Shi, Y., Ding, Y., Yang, S., 2015b. Cold signal transduction and its interplay with phytohormones during cold acclimation. Plant Cell Physiol. 56, 7–15. https://doi.org/10.1093/pcp/pcu115.

Smékalová, V., Doskočilová, A., Komis, G., Šamaj, J., 2014. Crosstalk between secondary messengers, hormones and MAPK modules during abiotic stress signalling in plants. Biotechnol. Ad 32, 2–11. https://doi.org/10.1016/j.biotechadv.2013.07.009.

Su, J., Zhang, M., Zhang, L., Sun, T., Liu, Y., Lukowitz, W., Xu, J., Zhang, S., 2017. Regulation of stomatal immunity by interdependent functions of a pathogen-responsive MPK3/MPK6 cascade and abscisic acid. Plant Cell 526–542. https://doi.org/10.1105/tpc.16.00577.

Teige, M., Scheikl, E., Eulgem, T., Dóczi, R., Ichimura, K., Shinozaki, K., Dangl, J.L., Hirt, H., 2004. The MKK2 pathyway mediates cold and salt stress signaling in *Arabidopsis*. Mol. Cell 15, 141–152. https://doi.org/10.1016/j.molcel.2004.06.023.

Wang, R., 2014. Gasotransmitters: growing pains and joys. Trends Biochem. Sci. 39, 227–232. https://doi.org/10.1016/j.tibs.2014.03.003.

Wilkinson, S., Clephan, A.L., Davies, W.J., 2001. Rapid low temperature-induced stomatal closure occurs in cold-tolerant *commelina communis* leaves but not in cold-sensitive tobacco leaves, via a mechanism that involves apoplastic calcium but not abscisic acid. Plant Physiol. 126, 1566–1578. https://doi.org/10.1104/pp.126.4.1566.

Xuan, A., Long, D., Li, J., Ji, W., Zhang, M., Hong, L., Liu, J., 2012. Hydrogen sulfide attenuates spatial memory impairment and hippocampal neuroinflammation in beta-amyloid rat model of Alzheimer's disease. J. Neuroinflamm. 9 https://doi.org/10.1186/1742-2094-9-202, 202–202.

Yang, G., Sun, X., Wang, R., 2004. Hydrogen sulfide-induced apoptosis of human aorta smooth muscle cells via the activation of mitogen-activated protein kinases and caspase-3. FASEB J. 18, 1782–1784. https://doi.org/10.1096/fj.04-2279fje.