Salinity stress from soil or irrigation water can significantly limit the growth and development of plants. Emerging evidence suggests that hydrogen sulfide (H$_2$S), as a versatile signal molecule, can ameliorate salt stress-induced adverse effects. However, the possible physiological mechanism underlying H$_2$S-alleviated salt stress in cucumber remains unclear. Here, a pot experiment was conducted with an aim to examine the possible mechanism of H$_2$S in enhancement of cucumber salt stress tolerance. The results showed that H$_2$S ameliorated salt-induced growth inhibition and alleviated the reduction in photosynthetic attributes, chlorophyll fluorescence and stomatal parameters. Meanwhile, H$_2$S increased the endogenous H$_2$S level concomitant with increased activities of D/L-cysteine desulfhydrase and β-cyanoalanine synthase and decreased activities of O-acetyl-L-serine(thiol)lyase under excess NaCl. Notably, H$_2$S maintained Na$^+$ and K$^+$ homeostasis via regulation of the expression of PM H$^+$-ATPase, SOS1 and SKOR at the transcriptional level under excess NaCl. Moreover, H$_2$S alleviated salt-induced oxidative stress as indicated by lowered lipid peroxidation and reactive oxygen species accumulation through an enhanced antioxidant system. Altogether, these results demonstrated that application of H$_2$S could protect cucumber seedlings against salinity stress, likely by keeping the Na$^+$/K$^+$ balance, controlling the endogenous H$_2$S level by regulating the H$_2$S synthetic and decomposition enzymes, and preventing oxidative stress by enhancing the antioxidant system under salinity stress.

Keywords: salt stress, hydrogen sulfide, Cucumis sativus L., lipid peroxidation, Na$^+$/K$^+$ balance, expression of SKOR gene
INTRODUCTION

Salinization of soil is pervasive throughout the globe, which is gradually becoming an earnest threat to world agriculture, affecting approximately 20% of arable irrigated land and consequently leading to the loss of United States $27.5 billion per annum (Abdel Latef et al., 2019). Salt stress is a major environmental factor that leads to significant inhibition of plant growth and decrease of productivity. Salt stress can greatly inhibit plant growth by decreasing biomass production and reducing the $P_{n}$ and $T_{r}$ (Chen et al., 2016). Chlorophyll fluorescence is considered a tool for interpreting stress tolerance in plants. Measurement of the chlorophyll fluorescence from PSII, the primary reactions of photosynthesis, provides an assessment of plant stress (Diao et al., 2014). Under adverse conditions, $F_{o}$ is an important indicator of damage to the PSII reaction center, while the $F_{v}$/$F_{m}$ and $F_{v}$/$F_{o}$ ratios reflect its maximum photoenergy conversion efficiency and its potential photochemical efficiency. The $F_{v}$/$F_{m}$ and $\Phi_{PSII}$ represent the primary and actual light energy conversion capacities, respectively. Any decreases in $F_{v}$/$F_{m}$ and $\Phi_{PSII}$ indicate decline in photochemical activity of PSII (Kumar and Parsad, 2015). In general, the $F_{v}$/$F_{m}$, $\Phi_{PSII}$ and qL parameters have been calculated to reflect photochemical quenching, while NPQ is a non-photochemical-quenching parameter (Zhang et al., 2009). The factor $q_{P}$ represents the reduced state of the primary electron acceptor QA (Kumar and Parsad, 2015). Under saline conditions, there is a general decrease in $q_{P}$ parameters ($F_{v}$/$F_{m}$ and $F_{o}$) and in the ETR, but increases in $q_{P}$, $\Phi_{PSII}$ and NPQ.

It is well known that salt stress results in the over-accumulation of $Na^{+}$ in plants cells, which competitively inhibit the uptake of $K^{+}$, thus leading to a deficiency of $K^{+}$ (Zhu, 2003; Munns and Tester, 2008). Plants have evolved varying mechanisms to adapt to salinity, including reducing $Na^{+}$ influx into the root, control of $Na^{+}$ xylem loading, $Na^{+}$ retrieval from the xylem, $Na^{+}$ recirculation in the phloem, $Na^{+}$ efflux from the root, intracellular compartmentation of $Na^{+}$ into the vacuoles, and $Na^{+}$ secretion from the leaf (Cui et al., 2011; Kronzucker and Britto, 2011). The best way to limit $Na^{+}$ accumulation in plants would be to reduce $Na^{+}$ influx into the root in the first place, leading to improved salt tolerance of crop plants. The SOS pathway is essential for salt stress tolerance and maintaining ion homeostasis in the cytoplasm (Zhu, 2002). For example, a plasma membrane Na+/H+ antiporter (SOS1) is able to remove excess $Na^{+}$ from cells (Feki et al., 2014). The $PM H^{+}$-ATPase also plays an important role in the regulation of ion homeostasis in the cytosol when plants encountered salt stress (Binzel, 1995). On the other hand, the prevention of $Na^{+}$-caused $K^{+}$ leakage is also critical for plant salt tolerance (Lai et al., 2014). $K^{+}$ channels or transporters are responsible for the $K^{+}$ transport into or out of root cells. Several reports showed that the outward-rectifying $K^{+}$ channel such as SKOR is involved in $K^{+}$ efflux (Lai et al., 2014). The SKOR is expressed in the root stele and responsible for $K^{+}$ release to the xylem (Gaymard et al., 1998).

Plants under salinity stress usually produce excessive amounts of ROS, such as $O_{2}$. $\cdot^{-}$, $H_{2}O_{2}$, and $H_{2}S$ (Yin et al., 2016). And the excessive accumulation of ROS is potentially harmful to proteins, DNA and lipids, leading to impairment of membrane integrity, enzyme inhibition and chlorophyll degradation. Therefore, ROS scavenging is important for plant survival and growth under salinity stress conditions. To combat with excessive accumulation of ROS, plants have evolved a protective strategy by activating antioxidant system, which includes non-enzymatic antioxidants, such as acid ASA and GSH, and enzymatic antioxidants, such as SOD (EC1.15.1.1), CAT (EC 1.11.1.6), POD (EC 1.11.1.7), GPX (EC 1.11.1.9), APX (EC 1.11.1.11), and GR (EC 1.6.4.2). The functions of ASA and GSH are closely related to their redox states (Kocsy et al., 2001) and plants can adjust redox states of ASA and GSH by modulating their regeneration and biosynthesis.

For a long time, $H_{2}S$ has been considered as merely a toxic by-product of cellular metabolism, but nowadays it is emerging as a novel gaseous signal molecule, which participates in seed germination, plant growth and development, as well as the acquisition of stress tolerance including cross-adaptation in plants (Li et al., 2016). $H_{2}S$ homeostasis is closely regulated by L-CD, EC 4.4.1.1, D-CD, EC 4.4.1.15, CAS, EC 4.4.1.9, OAS-TL, EC 2.5.1.47, and so on (Li et al., 2016). L-CD and D-CD can catalyze the degradation of L-$CD$ or D-$CD$, which respond to environment stress to promote stress tolerance. Wang et al. (2012) reported that $H_{2}S$ can enhance Medicago sativa tolerance against salinity via the NO pathway, which is also confirmed by Chen et al. (2015) in barley seedling. It is reported that $H_{2}S$ can coordinate regulation of the SOS pathway to overcome the deleterious effects of salt stress in strawberry.
(Christou et al., 2013). Deng et al. (2016) also confirmed that H$_2$S kept a lower Na$^+$ concentration via the regulation the SOS1 pathway to alleviate growth inhibition in wheat seedlings under NaCl stress. Subsequently, Lai et al. (2014) reported that endogenous H$_2$S can enhance salt tolerance by the establishment of redox homeostasis and preventing salt-induced K$^+$ loss in seedlings of Medicago sativa. In addition, H$_2$S can also synergistically regulate Na$^+/K^+$ balance, mineral homeostasis and oxidative metabolism in rice under excessive salt stress (Mostofa et al., 2015b). But these studies are commonly involved in one aspect, either Na$^+/K^+$ balance or antioxidation system, either in roots or leaves, and there are limited reports on the overall changes in morphology, photosynthesis, stomatal responses, ROS accumulation and the correlation between changes in the leaves and roots following exogenous H$_2$S treatment. Furthermore, the underlying mechanisms through which H$_2$S regulates salt tolerance, especially the changes in H$_2$S homeostasis, are still elusive, requiring in-depth analysis at the physiological and biochemical levels. Cucumber (Cucumis sativus L.) is an economically important crop as well as a model plant for systematic investigations on many aspects.

In this study, a pot experiment was conducted with an aim to evaluate the possible mechanism by which H$_2$S enhances tolerance of salinity stress in the important vegetable crop cucumber, with particular emphasis on maintenance of the Na$^+/K^+$ balance, and regulating of H$_2$S metabolism and oxidative stress response.

**MATERIALS AND METHODS**

**Plant Culture and Treatments**

Under salt stress, the morphological characters and physiological indexes of sensitive cultivars are more obvious than tolerant cultivars. When the sensitive plants were treated with H$_2$S, the ameliorative effects of H$_2$S will be easy to judge. Therefore, the sensitive cucumber (C. sativus L.) cultivar “Chunxiaqiwang” was used in our experiments. Uniform seeds were sterilized in 10 % (v/v) sodium hypochlorite solution for 5 min followed by washing three times with distilled water. Sterilized seeds were germinated on moist filter paper in petri dishes at 25°C in the dark. After germination, seedlings were transplanted into perlite-filled plastic pots (240 mL, 65 × 45 × 70 mm, a seedling per pot) and watered with Hoagland’s solution. 10 mL Hoagland’s solution was applied for every other day after germination, which contained 5 mM KNO$_3$, 5 mM Ca(NO$_3$)$_2$, 1 mM NH$_4$H$_2$PO$_4$, 2 mM MgSO$_4$, 10 µM MnSO$_4$, 50 µM H$_3$BO$_3$, 0.7 µM ZnSO$_4$, 0.2 µM CuSO$_4$, 0.01 µM (NH$_4$)$_2$Mo$_7$O$_{24}$ and 70 µM Fe-EDTA-Na$_2$. The seedlings of cucumber were maintained in a controlled growth chamber with a light/dark regime of 14/10 h, relative humidity of 70%, temperature of 25°C and a PAR of 800 µmol.m$^{-2}$.s$^{-1}$. When the first true leaf emerged, uniform and healthy seedlings were selected and divided into three groups for the following treatments. (i) Hoagland’s nutrient solution (as Control); (ii) Hoagland’s nutrient solution + 200 mM NaCl (as NaCl); (iii) Hoagland’s nutrient solution + 200 mM NaCl + 5 or 10, or 15 or 20 mM NaHS (as NaCl + NaHS). In our preliminary experiment, the phenotypic characteristics of plants with treated by H$_2$S alone were no significant difference compared with the control group (data not shown). Thus there is no only H$_2$S treatment group in our study. NaHS is commonly used as an H$_2$S donor since it dissociates to water produce HS$^-$ and Na$^+$, and then combination of HS$^-$ with H$^+$ produces H$_2$S (Lin et al., 2012). NaHS is responsible for induction of stress tolerance, but not Na$_2$S, Na$_2$SO$_3$, NaHSO$_4$, Na$_2$SO$_3$, NaHSO$_3$, or CH$_3$COONa (Deng et al., 2016). Each treatment was replicated three times under the same experimental conditions. The NaHS was purchased from Sigma (St Louis, MO, United States) and used as H$_2$S donor. The remaining treatment solution, which was not fully absorbed by plants, was removed and 10 mL fresh treatment solution was irrigated every other day at 9:00 AM during the whole culture process. After 7 days of treatment, the parameters of photosynthesis and chlorophyll fluorescence were determined using the first true leaf of seedlings with different treatments. And finally, at least 24 seedlings per treatment group were harvested and weighed separately to determine various physiological and biochemical parameters, while other samples were immediately frozen in liquid N$_2$ and then stored in a −80°C freezer unit until use.

**Morphological Assessment**

Root length, seedling length (including aboveground and underground parts) and leaf area index were determined after 7 days of treatment. Leaf area index was measured using graph paper according to the method of Gupta et al. (2017). Plant roots were gently removed from the perlite, and then washed three times with deionized water to remove adhered perlite particles. After absorbing moisture from the root surface with a clean filter paper, the fresh weights of root and shoot were recorded, respectively. Shoot dry weight and root dry weight were, respectively, recorded after drying the same plants in an oven at 60 °C until the weight became constant. Survival rate (%) was calculated as the number of survival seedlings / number of total seedlings. The rate of second leaf expansion (%) was calculated as the number of seedlings with appearance of the second leaf / number of total seedlings.

**Measurements of Photosynthesis, Chlorophyll Fluorescence, and Chlorophyll Content**

The first true leaf was chosen to determine the parameters of photosynthesis, chlorophyll fluorescence and chlorophyll Content. And the $P_m$ and $T_r$ were measured using an LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, United States). The leaf chlorophyll fluorescence parameters were measured using a portable chlorophyll fluorometer (O.S.-30p+, Opti-Science, Inc., Tyngsboro, MA, United States). The $F_o$ emission was determined on dark-adapted leaves. The $F_m$ was obtained during a subsequent saturating light pulse. A second saturating pulse of white light was imposed to determine the $F_{m'}$. The $F_{m}$ was determined by illuminating the leaf with far-red light for 3 s. The fluorescence parameters were calculated according to Hu and Yu (2014): maximal
quantum yield of PSII photochemistry, $F_v/F_m = (F_m - F_o)/F_m$; effective quantum-use efficiency of PSII in light-adapted state, $F_v/F_m' = (F_m' - F_o)/F_m'$; quantum yield of PSII photochemistry, $\Phi_{PSII} = (F_m' - F_o')/F_m'$; $qP = (F_m' - F_o')/(F_m' - F_o)$; and NPQ = $(F_m' - F_m)/F_m'$. The contents of Chlorophyll a ($C_a$), chlorophyll b ($C_b$), and $C_a + C_b$ were measured on fresh leaves as described by Lichtenthaler and Wellburn (1983).

**Analysis of Stomatal Parameters by Scanning Electron Microscope**

The first true leaves were selected to measure the stomatal opening by scanning electron microscopy (S-3400N; Hitachi, Tokyo, Japan). The leaf epidermis and stomata were observed and photographed under 15 kV. The diameter of the stomata was measured by Motic Images Advanced 3.2 (Notic China Group Co., Ltd., China) software at 300× magnification.

**Analysis of Electrolyte Leakage and Malondialdehyde Content**

Membrane integrity was evaluated in the leaves and roots by measuring electrolyte leakage. Fresh leaf or root samples (50 mg) were cut in small pieces followed by repeated washings with double distilled water. And then the samples were incubated at 28°C for 2 h in test tubes containing 10 mL of double distilled water for determining EC1 using a conductivity meter (Lei-Ci, DDSJ-308F, Shanghai, China). The same samples were again kept in water bath at 100°C for 20 min and the EC2 was recorded at room temperature. The electrolyte leakage was calculated as electrolyte leakage (%) = (EC1/EC2) × 100%. MDA content in the leaves or roots was measured by TBA reaction according to Shi et al. (2014) with modifications. Leaves or roots tissue (0.5 g) was homogenized in 10 mL of 0.1% (w/v) TCA, and the extract was centrifuged at 5,000 × g for 10 min at 4°C. To measure MDA, 1.5 mL of the supernatant was added into 1.5 mL of 0.5% (w/v) TBA made in 5% TCA. The mixture was heated at 100°C for 20 min and then quickly cooled in an ice bath. After centrifuging at 7,888 × g for 10 min, the absorbance of the supernatant was measured at 450 nm, 532 nm, and 600 nm, respectively. The MDA content of leaves or roots (per 0.5 g, FW) was calculated using the formula: $C (= nmol \cdot g^{-1} \cdot FW) = 20 \times [6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}]$.

**Determination of Endogenous $O_2^{•-}$ and $H_2O_2$ Levels**

The production of $O_2^{•-}$ was determined using the protocol described by Jiao et al. (2011) with some modifications. Samples (0.5 g) were homogenized in an ice bath with 1.2 mL of phosphate buffer (pH 7.8) and centrifuged at 5,000 × g for 10 min at 4°C. The supernatant was reacted with 1 mL of hydroxylamine hydrochlorides for 1 h, then 1 mL of p-aminobenzenesulfonic acid and 1 mL of α-naphthylamine were added. The solution was kept at 25°C for 20 min. The optical density value of the solution was measured with a spectrophotometer at 530 nm using NaNNO₂ as the standard curve and the corresponding calibration curves was $Y = 552.16 \times X + 4.365$ ($R^2 = 0.9903$) $H_2O_2$ was measured spectrophotometrically according to Jiang et al. (2012). Roots and leaves (0.2 g) were homogenized in an ice bath with 1 mL of 0.1% TCA and centrifuged at 12,000 × g for 20 min at 4°C. The supernatant was used for measuring $H_2O_2$ content. The reaction mixture consisted of 0.5 mL of the extracted supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. The reaction was developed for 1 h in darkness before the absorbance was measured at 390 nm. The blank was 0.1% TCA in the absence of root extract. The amount of $H_2O_2$ was calculated using a standard curve prepared with known concentrations of $H_2O_2$. The corresponding calibration curves was $Y = 82.69 X - 1.11$ ($R^2 = 0.9959$).

**Determination of Endogenous $H_2S$ Level and the Activities of L-CD, D-CD, CAS, and OAS-TL**

Endogenous $H_2S$ content was determined by the formation of methylene blue from dimethyl-p-phenylenediamine in $H_2SO_4$ according to the method described previously (Zhang et al., 2008). Samples (0.5 g) were extracted in 1 mL phosphate buffer solution (50 mM, pH 6.8) containing 0.2 M ASA and 0.1 M EDTA. Subsequently, 1 M HCl (0.5 mL) was added to the homogenate in a test tube for releasing $H_2S$, and 0.5 mL of 1% (w/v) zinc acetate was used to absorb $H_2S$. After 30 min reaction, 0.3 mL 5 mM $N,N$-dimethyl-p-phenylenediamine dihydrochloride dissolved in 3.5 mM $H_2SO_4$ was injected into the trap. Then 0.3 mL of 50 mM ferric ammonium sulfate in 100 mM $H_2SO_4$ was injected into the trap. The amount of $H_2S$ in zinc acetate traps was determined spectrophotometrically at 670 nm, after leaving the mixture for 15 min at room temperature. Solutions with different concentrations of Na$_2$S were prepared, treated in the same way as the assay samples and were used for the quantification of $H_2S$.

Samples (0.3 g) were ground with a mortar and pestle in liquid nitrogen and soluble proteins were extracted with 1.5 mL of cold extraction buffer containing 20 mM Tris–HCl (pH 8.0), 0.1% (w/v) DTT and 0.2% (w/v) sodium ascorbate. The homogenate was centrifuged at 13,000 × g for 15 min. The resulting supernatant was used for the determination of the activities of L/D-CD, CAS, and OAS-TL. Total L-CD activity was determined by the release of $H_2S$ from L-cysteine as described in Riemenschneider et al. (2005) with minor modification. The assay contained in a total volume of 1 mL: 0.1 mL of 10 mM L-cysteine, 0.8 mL 100 mM Tris–HCl (containing 2.5 mM DTT, pH 9.0) and 0.1 mL of protein solution. After incubation for 60 min at 30°C, the reaction was terminated by adding 0.1 mL of 30 mM FeCl₃ dissolved in 1.2 N HCl and 0.1 mL of 20 mM N, N-dimethyl-p-phenylenediamine dihydrochloride dissolved in 7.2 N HCl. The formation of methylene blue was determined at 670 nm, and known concentrations of Na$_2$S were used in the calibration curve. D-CD activity was determined in the same way as L-CD activity except with D-cysteine instead of L-cysteine and the pH of the Tris–HCl buffer was 8.0. One unit of enzyme activity is defined as the amount of enzyme that produces 1.0 mmol/min $H_2S$ under the stated assay conditions and expressed as U g$^{-1}$ FW. The total OAS-TL activity was determined following the method described.
Bloem et al. (2004). CAS activity was measured by the release of H$_2$S from L-cysteine in the same way as L-CD activity with the following modifications: the 1.0 mL volume of reaction mixture contained 0.1 mL of 10 mM L-cysteine, 0.7 mL 100 mM Tris–HCl (pH 9.0), 0.1 mL of 7.5 mM KCN and 0.1 mL of protein solution, as described in Meyer et al. (2003).

### Determination of Na$^+$ and K$^+$ Content and Na$^+$/K$^+$

Dried plant material (200 mg) was subjected to acid digestion in 10 mL digestion mixture [HClO$_4$: HNO$_3$: H$_2$SO$_4$ (5: 1: 1, v/v)] on a hot plate (120$^\circ$C) until the volume was reduced to 1 mL. After diluting with double distilled water, the Na$^+$ and K$^+$ contents were estimated using the flame photometer (Horneck and Hanson, 1998).

### Extraction of Total RNA and Real-Time RT-PCR Analysis

To study the effect of NaHS treatment on the expression of the SOS1, SKOR, and PM H$^+$-ATPase genes in cucumber leaves and roots in response to salt stress, 18-day-old seedlings treated with either Hoagland’s nutrient solutions (control group), 200 mM NaCl, or 200 mM NaCl + 15.0 µM NaHS for a week were harvested. Total RNA of cucumber leaves or roots (100 mg) was isolated by grinding with mortar and pestle in liquid nitrogen to a fine powder and using TriZol Reagent (Invitrogen) in accordance with the manufacturer’s protocol. The total RNA samples were treated with RNAase-free DNase (TaKaRa Bio Inc., Dalian, China) to eliminate traces of DNA, followed by quantification using the NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, United States). Total RNA (2 µg) was reverse-transcribed using an oligo d (T) primer (TaKaRa Bio Inc., Dalian, China) and the cycling conditions of denaturation at 95$^\circ$C for 5 min, followed by 40 cycles of denaturation at 95$^\circ$C for 15 s, annealing at 60$^\circ$C for 30 s, and extension at 72$^\circ$C for 15 s. Using specific primers (Supporting information, Supplementary Table S1), the expression levels of the genes were presented as values relative to the corresponding control samples under the indicated conditions, with normalization of data to the geometric average of internal control gene GAPDH (a housekeeping genes). Three independent replicates were performed for each sample. The comparative threshold cycle (Ct) method was used to determine the relative amount of gene expression. Relative gene expression levels were calculated via the 2$^{-\Delta\Delta Ct}$ method. The relative gene expression was determined as previously described by Livak and Schmittgen (2001).

### Measurements of ASA, GSH, SOD, CAT, GR, and POD

The ASA content was determined using the DCIP method. Samples (0.50 g) were homogenized in 3.0 mL of 2 % oxalic acid supplemented with 0.50 mL of 30 % ZnSO$_4$ and 0.50 mL of 15% K$_4$Fe(CN)$_6$·3H$_2$O. The volume of the extract was adjusted to 10.0 mL with 1% oxalic acid, followed by centrifugation at 10, 000 × g for 10 min. Then 3 mL of the supernatant was mixed with 2.0 mL of dye solution (0.25% DCIP and 0.20% NaHCO$_3$) and 3 mL of dimethylbenzene. The absorbance was read at 500 nm, and the content of ASA was calculated using a standard curve. Total GSH was estimated using a kit (Jiancheng Bioengineering Institute, Nanjing, China). The absorbance of GSH was measured at 420 nm according to manufacturer’s instructions. CAT activity was measured according to the methods described by Velikova et al. (2000) using 10 mM potassium buffer (pH 7.00), with 100 µL of enzyme extract, and 33 mM H$_2$O$_2$. H$_2$O$_2$ was assayed from the decrease in absorbance in optical density at 240 nm, and the activity was calculated using the extinction coefficient of 40 mM cm$^{-1}$ of H$_2$O$_2$. GR activity was determined by measuring the absorbance change at 340 nm due to oxidation of NADPH (Foyer and Halliwell, 1976). SOD and POD activities were assayed as described by Chen et al. (2013).

### Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS (version 21.0.0; IBM, Armonk, NY, United States). Different letters on the graphs or in the tables indicated that the mean values were statistically different at the $P < 0.05$ level.

### RESULTS

**H$_2$S Alleviates NaCl-Induced Inhibition of Cucumber Plant Growth**

The morphology, survival rate, and expansion rate of the second leaf were determined after cucumber seedlings exposed to 200 mM NaCl for 7 days. Under salt stress, most seedlings began to show leaf wilting, lodging and inhibition of the second leaves compared with control group (Supporting information, Supplementary Figure S1). In order to analyze the alleviation effect of NaHS on growth inhibition induced by NaCl in a dose-dependent manner, series of concentrations of NaHS were added to the nutrient solution. In cucumber seedlings, 15 or 20 µM NaHS significantly alleviated the inhibition of plant growth during salt stress (Supplementary Figure S1A). Salinity treatment significantly ($P < 0.05$) reduced the survival rate by 89 %, the rate of expansion of the second leaf by 62 % (Supplementary Figures S1B,C) and significantly ($P < 0.05$) affected general growth (Figure 1A). Compared with the control, the shoot fresh weight, root fresh weight, seedling fresh weight, seedling total length, shoot dry weight, root dry weight, leaf area and root length were significantly ($P < 0.05$) reduced by 66%, 58%, 65%, 43%, 50%, 53%, 90%, and 48% under salt stress, respectively (Figures 1B–1). However, all growth parameters showed different degree ameliorating effects with application of different concentrations of NaHS (Figure 1 and Supplementary Figure S1). For example, the combination NaCl+15 µM NaHS significantly ($P < 0.05$) reduced shoot fresh weight, root fresh weight, seedling fresh weight, seedling total length, shoot dry weight, root dry weight, leaf area and root length by 28%, 40%, 31%, 14%, 17%, 42%, 41%, and 15%,
FIGURE 1 | Effects of NaHS treatment on the NaCl-induced changes in cucumber seedling growth (A). Plant growth was evaluated by the indexes of shoot fresh weight (B), root fresh weight (C), fresh weight of seedling (D), length of seedling (E), shoot dry weight (F), root dry weight (G), leaf area (H) and root length (I) in Cucumis sativus L. cv. Chunxiqiuwang grown under 200 mM NaCl. Each value is the mean of three biological replicates, and the vertical bars represent the standard errors. Values sharing the same lower case letters are insignificant as per Duncan's test at $P < 0.05$.

respectively, as compared to the control (Figures 1B–I). Thus, these results indicated that exogenous supplementation with 15 $\mu$M NaHS was the most ameliorating effect in boosting cucumber tolerance to NaCl stress.

Effects of H$_2$S on Photosynthesis Under Excess NaCl

In comparison with the control, a significant ($P < 0.05$) decline in the $P_n$ (by 14.3%) and a sharp decline in the $T_r$ (by 94%) were recorded in the leaves of salt-stressed cucumber plants (Table 1). The combination of NaCl and 15 $\mu$M NaHS showed a decline in the $P_n$ (by 2.4%) and $T_r$ (by 90.4%) compared with the control, respectively (Table 1). Nevertheless, 5 $\mu$M NaHS did not significantly affect the $T_r$ under salt stress. In addition, 20 $\mu$M NaHS alleviated the declines of $P_n$, but did not cause a significant change of $T_r$ under salt stress.

As shown in Table 1, salt stress significantly ($P < 0.05$) reduced $F_v/F_m$ (by 6%), $F_s/F_o$ (by 25%), $\Phi_{PSII}$ (by 7%) and $qP$ (by 12%) compared with the control, while 15 $\mu$M NaHS treatment reduced these by 0.5%, 4.5%, 2% and 3.1%, respectively, compared with the control. In contrast, comparing with the control, the parameters of $F_o$ and NPQ significantly ($P < 0.05$) increased by 39% and 78% under salt stress. As expected, 15 $\mu$M NaHS significantly reversed the increase in $F_o$ (by 10.4%) and NPQ (by 34.2%) under salt stress (Table 1).

Salt stress resulted in a decrease in the contents of the photosynthetic pigments $C_a$, $C_b$, $C_{a+b}$ and $C_{x+c}$ compared to the non-treated plants. Compared with the control, salt stress reduced $C_a$, $C_b$, $C_{a+b}$ and $C_{x+c}$ content by 38%, 37%, 36%, and 35%, respectively, while 15 $\mu$M NaHS treatment reduced $C_a$, $C_b$, $C_{a+b}$, and $C_{x+c}$ content by 11.2%, 8.7%, 7.8% and 15% (Table 1).

Effects of H$_2$S on the Stomatal Index Under Excess NaCl Conditions

To determine the effects of H$_2$S on stomata in cucumber, the abaxial leaf surface was observed by a scanning electron
Each value is the mean of three biological replicates. Values sharing the same lower case letters are insignificant as per Duncan’s test at \( P < 0.05 \).

microscope to analyze several stomatal indexes (Figures 2A–C). Salt stress induced stomatal closure and resulted in abnormal morphology of the epidermal and guard cells compared to the untreated control plants, whereas application of 15 \( \mu \text{M} \) NaHS significantly reversed these damages. Salt stress caused a noticeable reduction in stomatal opening rate by 67% (Figure 2D) and stomatal width by 74% (Figure 2E), and a significant increase in stomatal density by 41% (Figure 2F) and stomatal length by 36% (Figure 2G) compared with control plants. However, 15 \( \mu \text{M} \) NaHS treatment reduced stomatal opening rate by 30.8% (Figure 2D) and stomatal width by 52.8% (Figure 2E), and increased stomatal density by 12.6% (Figure 2F) and stomatal length by 12% (Figure 2G) compared with control plants. The diverse responses of the stomata indicated that there is co-ordination of the physiological (i.e., aperture) and morphological (i.e., stomatal density) changes induced by

### TABLE 1 | Effects of NaHS treatment on the \( P_n \), \( T_r \), \( F_0 \), \( F_v/F_m \), \( F_v/F_o \), \( \Phi_{PSII} \), NPQ, and \( q_P \) values and the contents of \( C_a \), \( C_b \), \( C_o+b \), and \( C_{a+c} \) in \textit{Cucumis sativus} L. cv. Chunxiaqiuwang grown under 200 \( \mu \text{M} \) NaCl.

| Index | Control | NaCl | NaCl+5 \( \mu \text{M} \) NaHS | NaCl+10 \( \mu \text{M} \) NaHS | NaCl+15 \( \mu \text{M} \) NaHS | NaCl+20 \( \mu \text{M} \) NaHS |
|-------|---------|------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| \( P_n \) (\( \mu \text{mol. m}^{-2}\text{s}^{-1} \)) | 8.037 ± 0.286a | 6.888 ± 0.095d | 7.427 ± 0.129c | 7.678 ± 0.113b | 7.848 ± 0.119ab | 7.443 ± 0.090c |
| \( T_r \) (\( \mu \text{mol. m}^{-2}\text{s}^{-1} \)) | 5.236 ± 0.205a | 0.291 ± 0.006c | 0.327 ± 0.028bc | 0.371 ± 0.023bc | 0.502 ± 0.019b | 0.427 ± 0.037bc |
| \( F_0 \) | 183.00 ± 8.08d | 253.50 ± 3.70a | 211.50 ± 5.74bc | 217.75 ± 9.78b | 204.25 ± 1.71c | 214.00 ± 6.27b |
| \( F_v/F_m \) | 0.801 ± 0.005a | 0.753 ± 0.009c | 0.775 ± 0.019b | 0.771 ± 0.009b | 0.797 ± 0.001a | 0.781 ± 0.008b |
| \( F_v/F_o \) | 4.097 ± 0.096a | 3.085 ± 0.136d | 3.761 ± 0.069bc | 3.642 ± 0.306c | 3.914 ± 0.033ab | 3.658 ± 0.085c |
| \( \Phi_{PSII} \) | 0.735 ± 0.004a | 0.685 ± 0.002c | 0.716 ± 0.004b | 0.710 ± 0.013b | 0.720 ± 0.004b | 0.718 ± 0.000b |
| NPQ | 0.175 ± 0.015d | 0.312 ± 0.007a | 0.292 ± 0.027ab | 0.288 ± 0.015ab | 0.266 ± 0.007bc | 0.242 ± 0.029c |
| \( q_P \) | 3.614 ± 0.076a | 3.173 ± 0.020b | 3.439 ± 0.018ab | 3.496 ± 0.226a | 3.503 ± 0.024a | 3.478 ± 0.060a |
| \( C_a \) (mg. g\(^{-1}\) FW) | 1.315 ± 0.120a | 0.812 ± 0.009d | 0.841 ± 0.005d | 0.924 ± 0.062cd | 1.168 ± 0.082b | 1.005 ± 0.016c |
| \( C_b \) (mg. g\(^{-1}\) FW) | 0.356 ± 0.039a | 0.224 ± 0.006bc | 0.235 ± 0.001c | 0.253 ± 0.017c | 0.325 ± 0.017ab | 0.299 ± 0.017bc |
| \( C_o+b \) (mg. g\(^{-1}\) FW) | 1.620 ± 0.160a | 1.036 ± 0.015d | 1.074 ± 0.009cd | 1.177 ± 0.079cd | 1.493 ± 0.099ab | 1.296 ± 0.003bc |
| \( C_{a+c} \) (mg. g\(^{-1}\) FW) | 0.307 ± 0.023a | 0.199 ± 0.003d | 0.206 ± 0.000cd | 0.220 ± 0.013cd | 0.261 ± 0.022b | 0.244 ± 0.019bc |

\( \text{FW} \) is fresh weight.
Effects of H₂S Treatment on Electrolytic Leakage, Lipid Peroxidation, and ROS Accumulation Under Excess NaCl

The exposure of the cucumber plants to salinity significantly (P < 0.05) increased electrolyte leakage by 1.1 fold in leaves (Figure 3A) and by 87.2% in roots (Figure 3B), and increased the MDA content by 45% in leaves (Figure 3C) and by 1.0 fold in roots (Figure 3D) compared with the control. However, treatment of salt-stressed plants with 15 µM NaHS resulted in an increase in electrolyte leakage by 27.6% in leaves and by 46% in roots (Figures 3A,B) and an increase in the MDA content by 21% in leaves and by 5.4% in roots as compared to the control (Figures 3C,D). These results indicated that NaHS clearly reduces cell membrane injury.

Salt stress treatment did not change the O₂•− accumulation in cucumber leaves (Figure 3E), but it did increase by nearly 2.1 fold in roots as compared to control plants (Figure 3F). While different concentration of NaHS treatment increased the accumulation of O₂•− in cucumber leaves and roots as compared to the control (Figures 3E,F), Salt stress caused a dramatic increase in H₂O₂ by 1.92 fold in leaves (Figure 3G) and 1.04 fold in roots (Figure 3H) compared with the control. Treatment with 15 µM NaHS increased the H₂O₂ by 21.7% in leaves (Figure 3G) and 19.7% in roots compared with the control (Figure 3H). These results indicated that exogenous application of NaHS suppresses ROS accumulation, thereby protecting cucumber plants from NaCl-induced oxidative stress.

Effects of NaHS Treatment on Endogenous H₂S Levels and H₂S Metabolism Under Excess NaCl

In comparison with the control, a sharp decline in the content of endogenous H₂S was recorded in leaves (by 49%) (Figure 4A) and roots (by 45%) (Figure 4B) of salt-treated cucumber plants, whereas 15 µM NaHS treatment improved the content of endogenous H₂S by 9.9% in leaves (Figure 4A) and by 9.1% in roots (Figure 4B) compared with the control. Compared with the control, salt stress significantly (P < 0.05) decreased the activity of L-CD by 19% in leaves (Figure 4C) and 12% in roots (Figure 4D). The same change was found in the activity of D-CD after salt stress, which reduced by 13% in leaves (Figure 4E) and 33 % in roots compared with the control (Figure 4F). As expected, these changes were reverted by different concentrations of NaHS treatment. For example, treatment with 15 µM NaHS significantly (P < 0.05) improved the activities of L-CD (38.7% in roots) and D-CD (by 52.7% in leaves and 4.6 fold in roots) compared with the control (Figures 4C-F). These results indicated that NaHS treatment significantly enhanced the activities of L-CD and D-CD in cucumber seedlings under salt stress.

As for the activity of CAS, a completely opposite trend was found between leaves and roots. Salt stress significantly (P < 0.05) improved the activity of CAS in leaves by 19% (Figure 4G) and reduced it by 43% in roots (Figure 4H) compared with the control. Application of 5 or 10 µM NaHS enhanced the activity of CAS by 9.4% or 8.9% in leaves, in contrast, 15 or 20 µM NaHS reduced it by 31.7% or 22.8% compared with the control. In the roots, 5~20 µM NaHS all reduced the activity of CAS in different degree compared with the control group (Figures 4G,H). In addition, the OAS-TL activity significantly (P < 0.05) increased in both leaves (by 65%) (Figure 4I) and roots (43%) (Figure 4J) under salt stress. Similarly, these effects were reverted by different concentrations of NaHS as compared to the plants receiving salinity treatment alone. For example, 15 µM NaHS treatment reduced the activity of OAS-TL by 42% in leaves and 2.4% in roots as compared to the control (Figures 4I,J).

Effects of H₂S on Na⁺ and K⁺ Homeostasis Under Excess NaCl

In plants, maintaining a lower Na⁺/K⁺ ratio is critical to its ability to tolerate salt stress. Compared with the control, a sharp increase in the Na⁺ content was observed in leaves (by 2.13 fold) and roots (1.35 fold) under salt stress (Figures 5A,B). On the contrary, a significant decrease in the K⁺ content was recorded in leaves (by 30%) and roots (by 41%) under salt stress as compared to the control (Figures 5C,D). Accordingly, the Na⁺/K⁺ ratio significantly increased in leaves (by 3.4 fold) and roots (by 3 fold) of salt-treated plants than the control (Figures 5E,F). As expected, treatment with 15 µM NaHS improved the Na⁺ content in leaves by 1.9 fold and roots by 1.0 fold, and reduced the K⁺ content in leaves by 17.8% and roots by 28% compared with the control, indicating that 15 µM NaHS significantly reversed the increase of Na⁺ and the decrease of K⁺ under salt stress. In addition, the Na⁺/K⁺ ratio was significantly lower in the NaHS-treated plants when compared with the plants treated with salt alone (Figures 5E,F). Thus, H₂S eases excessive Na⁺ uptake and maintains the Na⁺/K⁺ homeostasis at the cellular level in cucumber plants under salt stress.

Effects of H₂S on Expression of PM H⁺-ATPase, SOS1, and SKOR Under Excess NaCl

To further characterize the effect of H₂S on the modulation of Na⁺/K⁺ homeostasis in cucumber seedlings upon salt stress, the transcript levels of PM H⁺-ATPase, SOS1 and SKOR were analyzed. Real-time RT-PCR analysis showed that NaCl treatment resulted in the significant decreases in the transcripts levels of PM H⁺-ATPase by 66.9% (Figure 6A), SOS1 by 42.7% (Figure 6C) and SKOR by 72.4% (Figure 6E) in cucumber leaves compared with the controls, respectively. In contrast, the relative expression of PM H⁺-ATPase, SOS1 and SKOR were significantly increased by 72.6% (Figure 6B), 2.3 fold (Figure 6D) and 1.17 fold (Figure 6F) in the roots under NaCl stress compared with the control. The NaCl-induced increases in PM H⁺-ATPase, SOS1 and SKOR transcripts were significantly reversed by 15 µM NaHS treatments in the cucumber roots. As shown in Figure 6, compared with the control, treatment with 15 µM NaHS revealed decreased
FIGURE 3 | Effects of NaHS treatment on the NaCl-induced changes of electrolyte leakage in leaf (A) and root (B), MDA content in leaf (C) and root (D), \( \text{O}_2^- \) in leaf (E) and root (F), and \( \text{H}_2\text{O}_2 \) content in leaf (G) and root (H) of Cucumis sativus L. cv. Chunxiaqiwang. Each value is the mean of three biological replicates, and the vertical bars represent the standard errors. Values sharing the same lower case letters are insignificant as per Duncan’s test at \( P < 0.05 \).
the relative expression of \( \text{PM} \ H^+\text{-ATPase} \), \( \text{SOS1} \) and \( \text{SKOR} \) by 57.1%, 59.5% and 75.1% in leaves, respectively. On the contrary, treatment with 15 \( \mu \text{M} \) NaHS+NaCl improved the relative expression of \( \text{PM} \ H^+\text{-ATPase} \), \( \text{SOS1} \) and \( \text{SKOR} \) by 2.1 fold, 2.59 fold and 3.21 fold in leaves, respectively, as compared to the control.
Effects of H$_2$S on the Antioxidant System

To evaluate the role of H$_2$S in mediating salt-induced antioxidant system, the levels of non-enzymatic antioxidants (ASA and GSH) and the activities of antioxidant enzymes (SOD, CAT, GR, and POD) were analyzed in leaves and roots of cucumber plants. As show in Table 2, compared with the control, the contents of ASA and GSH and the activity of GR were significantly increased by 98%, 73%, and 61% in the leaves, but they were decreased by 88%, 23%, and 2.25 fold, in the roots under NaCl treatment. However, application of exogenous NaHS ameliorated the effect...
of salt stress. For example, 15 µM NaHS treatment significantly increased the ASA content by 42.6% and the GR activity by 9.1%, and decreased GSH by 44% in the leaves as compared to the control. On the contrary, 15 µM NaHS treatment decreased the ASA content by 23.9% and the GR activity by 27.8%, and increased GSH by 48.7% in the roots as compared to the control. Compared with the control, SOD activity was increased under salt stress in the leaves by 23% and roots by 43%. Application of NaHS showed a synergistic effect resulting in decreased SOD activity in the leaves and roots under NaCl stress. The application 15 µM NaHS significantly decreased SOD activity in the leaves by 19% and roots by 15% as compared to NaCl stress alone.
The NaCl treatment significantly decreased the activity of CAT in the leaves by 6% and roots by 59% as compared to control, but increased the POD activity in the leaves by 25% and roots by 10%. Application of NaHS reversed these changes in the leaves and roots induced by salt stress.

**DISCUSSION**

In plants, some small biological molecules, such as phytohormones and signal molecules could be considered as a powerful tool in modifying plants' adaptability against adverse environment (Mostofa et al., 2015a). By exogenously application of the H₂S donor NaHS, numerous results demonstrated that H₂S participates in plant adaptive responses against multiple abiotic stresses (Lai et al., 2014). Ameliorative effects of H₂S treatment are a trending topic among researchers that work on abiotic stress in plants, accordingly there are various studies in the literature that investigate how this molecule increase plant stress tolerance. However, most of the studies are limited to a certain topic such as only photosynthesis or Na⁺ and K⁺ homeostasis or antioxidant defense. A comprehensive study that investigates several issues in the same experimental setup and provides evidence on how H₂S alleviates detrimental effects of salt stress is still limited. Our hydroponically based study aids in understanding how plants respond under NaCl-toxicity and how the use of H₂S is capable of reverting this toxicity.

**H₂S Alleviates NaCl-Induced Inhibition of Plant Growth**

Results showed that salinity stress inhibited plant growth performance as judged by decreased shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, leaf area and root length compared with control, but this inhibition was significantly alleviated by H₂S donor NaHS (Figure 1). Similarly, the reversion of salt stress responses by H₂S treatment are a trending topic among researchers that H₂S induces a toxicity response. In our study, the capacity of H₂S to enhance salt tolerance firstly increased and then decreased with the concentration improvement. Ameliorative effects of phenotypes were seen from 5 to 15 µM NaHS, but plants exhibited slight toxicity under the 20 µM NaHS treatment. It may be that excessive high levels of H₂S might lead to the ROS over-production, which further leads to the inhibition of the mitochondrial electron transport chain (Mancardi et al., 2009). In our study, 20 µM NaHS treatment indeed caused the ROS over-production and cell membrane injury than 15 µM NaHS as judged by the parameters of electrolyte leakage, MDA, O₂•− and H₂O₂ (Figure 3). Hence, the use of H₂S is a double-edged sword, and the maintenance of a suitable level of intracellular H₂S is key conferring salt tolerance in cucumber seedlings.
**H$_2$S Alleviates the NaCl-Induced Declines of Photosynthetic Attributes**

In this study, the $F_v/F_m$ was significantly decreased under salt stress, and they were remarkably improved with addition of 15 $\mu$M NaHS (Table 1). Similarly, the results of Christou et al. (2013) showed that 100 mM NaCl stress significantly decreased the values of $F_v/F_m$, while 100 $\mu$M NaHS significantly reversed the decline of $F_v/F_m$. In addition, a phenomenon that H$_2$S prevented the reduction of photosynthetic pigments $C_a$, $C_b$, $C_{a+b}$, and $C_{x+c}$ induced by salt stress was also observed in our study (Table 1). The results are consistent with these of Mostofa et al. (2015b), who reported that H$_2$S improved overall growth and biomass of salt-stressed rice plants which could be attributed to its role in protecting Chl$_a$, Chl$_b$ and Cx+c from salt-induced damage. As the photosynthetic parameters $P_n$ and $T_r$, our results showed that H$_2$S also alleviated the decrease in the photosynthetic parameters $P_n$ and $T_r$. These results are consistent with these of Dawood et al. (2012), who reported that H$_2$S reduced the Aluminum-induced inhibition of $P_n$, $G_s$ and $T_r$. Another study also demonstrated a similar result that NaHS treatment increased the $P_n$ and $G_s$ values in *Spinacia oleracea* seedlings under drought (Chen et al., 2016). Together these results suggest that H$_2$S plays a role in plant photosynthesis.

**Exogenous H$_2$S Changes Endogenous H$_2$S Accumulation Under Excess NaCl**

Earlier investigations demonstrated that hypoxia, salt and Cd stress induce notable increases in endogenous H$_2$S production in different plant species, such as pea, strawberry, alfalfa and Chinese cabbage (Cheng et al., 2013; Christou et al., 2013; Lai et al., 2014; Zhang et al., 2015). The activation of endogenous H$_2$S level after stress treatments indicated that H$_2$S might also be an important secondary messenger of stress sensing which in turn modulated plant physiological changes and downstream gene expressions (Christou et al., 2013). Surprisingly, a remarkable decline in endogenous H$_2$S production was observed in cucumber leaves and roots upon treatment with 200 mM NaCl for 7 days in our experiment. Further investigation is still required to detail the reason. Although different kinds of environmental stress factors caused differential dynamic changes in endogenous H$_2$S metabolism, exogenously applied NaHS, a well-known H$_2$S donor, all greatly enhanced H$_2$S concentration.

![FIGURE 7 | A proposed model for H$_2$S-mediated salt stress responses in cucumber. This model depicting how H$_2$S regulates the enhanced salt tolerance by mediating maintaining the Na$^+$/K$^+$ balance and regulating endogenous H$_2$S metabolism and oxidative stress response. Under salt stress, on the one hand, the application of NaHS reversed the expression up-regulation of PM H$^+$-ATPase, SOS1, and SKOR in roots to keep Na$^+$ and K$^+$ balance. On the other hand, the application of NaHS increase L/D-CD activity and decrease OAS and OAS-TL activities to regulating endogenous H$_2$S metabolism. Additionally H$_2$S altered the accumulation of antioxidants and the activities of antioxidant enzymes, leading to reduced ROS accumulation and membrane lipid peroxidation. The red arrow indicated up-regulation or increase and the green arrow indicated down-regulation or decrease in this model.](image-url)
Generally, synthesize H$_2$S in plants is associated with the enzymes L-CD, D-CD, OAS-TL or CAS, often in response to environmental stress, leading to accumulation of endogenous H$_2$S and the acquisition of stress tolerance. The activities of L-CD in leaves and D-CD in both roots and leaves all showed a significantly decreased after salt stress treatment as compared to the control (Figures 4C–F). Lai et al. (2014) found that NaCl could induce L-CD in Medicago sativa roots, while the total activity of D-CD was not significantly altered. Meanwhile the expression of L-CD and D-CD at the transcripts level were strongly induced by drought stress in Arabidopsis thaliana (Jin et al., 2011). In our study, exogenously applied 15 µM NaHS significantly enhanced the activities of D-CD in both roots and leaves and L-CD in roots compared with the control or salt stress group (Figures 4D–F). However, Cheng et al. (2013) found that exogenously applied NaHS decreased the total activity of L-CD and D-CD in Pisum sativum L. seedlings under hypoxia stress. In addition, NO could induce the activities of to L-CD, OAS-TL and CAS regulate the endogenous H$_2$S biosynthesis in maize roots (Peng et al., 2016). In our study, salt-stress significantly improved the activity of CAS in leaves and OAS-TL in leaves and roots and significantly reduced the activity of CAS in roots (Figures 4G–J). This is also in line with a report of Cheng et al. (2013), and their results showed that exogenously applied NaHS enhanced the activity of CAS and OAS-TL in Pisum sativum L. seedlings under hypoxia stress (Cheng et al., 2013).

H$_2$S Maintains Na$^+$ and K$^+$ Homeostasis by Regulating Expression of PM H$^+$-ATPase, SOS1, and SKOR Genes Under Excess NaCl

Maintenance of Na$^+$ and K$^+$ ion homeostasis is critically important for plants to survive under salt stress. Our study provided firm evidence that H$_2$S prevented Na$^+$ uptake and improved K$^+$ uptake in both leaves and roots, thereby restraining the salt-induced decrease of the Na$^+$/K$^+$ ratio (Figure 5). Similarly, Lai et al. (2014) observed that the biologically beneficial role of NaHS was due to its specific ability to retain K$^+$, thus preventing the increase of the Na$^+$/K$^+$ ratio in stellar cells under salt stress. In addition, Christou et al. (2013) showed that H$_2$S regulates the expression of SOS pathway genes and kept the balance of Na$^+$ and K$^+$ in strawberry plants under salt stress. Deng et al. (2016) also found that H$_2$S significantly improved the salt tolerance of wheat seedlings by regulating the membrane-bound translocation proteins of the SOS1 pathways to increase Na$^+$ extrusion and decrease Na$^+$ uptake. Interestingly, salt stress induced the up-regulation of PM H$^+$-ATPase, SOS1 and SKOR at the transcript levels in roots compared with the control, but a completely opposite trend in leaves. This is also in line with a report of Wang et al. (2013), and their results showed that the expression of CsSOS1 increased in roots and decreased in leaves with the concentration of NaCl increasing in cucumber. Perhaps the root irrigation treatment method in this study affected the expression of genes or some signal first in roots. In our study, H$_2$S alleviated the NaCl-induced increases in PM H$^+$-ATPase expression in roots. It has been known that NaCl stress also up-regulates the expression of genes encoding PM H$^+$-ATPase in roots (Shi et al., 2000). Moreover, it is well known that PM H$^+$-ATPase can sustain an H$^+$ gradient by promoting Na$^+$ efflux and H$^+$ influx to drive Na$^+$/H$^+$ antiport across the plasma membrane. It was also found that the administration of NaHS effectively prevented the NaCl-triggered K$^+$ efflux in the mature zone of alfalfa seedling roots, which might be partially related to the SKOR channel (Lai et al., 2014). These studies suggested that salt-tolerant need plants have an ability to prevent transport of Na$^+$ from roots to shoots and an improved ability to exclude Na$^+$ from the roots (Takahashi et al., 2007a,b).

H$_2$S Regulates the Antioxidant System and Reduces Oxidative Stress and Membrane Lipid Peroxidation Under Excess NaCl

Plants suffering from NaCl toxicity often exhibit symptoms associated with oxidative stress and membrane lipid peroxidation, which can result in accumulation of ROS and MDA (Hossain et al., 2005). In this study, high salinity triggered overproduction of O$_2$•− and H$_2$O$_2$, increased the electrolyte leakage, and caused accumulation of MDA in both cucumber leaves and roots (Figure 3). H$_2$S alleviated the salinity-induced oxidative damage, as evident by reduced levels of O$_2$•−, H$_2$O$_2$, MDA and electrolyte leakage in both cucumber leaves and roots (Figure 3). This is in line with a report of Mostofa et al. (2015b), and their results showed that H$_2$S reduced the salt-induced increase in the levels of O$_2$•−, H$_2$O$_2$ and MDA in rice. Similar results were also reported by Chen et al. (2016), who demonstrated that NaHS treatment counteracted the accumulation of ROS and increased the MDA content of Spinacia oleracea L. seedlings under drought. The balance between ROS production and antioxidant defense determines the extent of oxidative damage within the plant. In our study, NaHS treatment significantly alleviated the improvement of ASA and GSH contents and SOD, GR, and POD activities in leaves of cucumber under salt stress (Table 2). In cucumber roots, H$_2$S also successfully counteracted the decline in the content of ASA and GSH and the activities of CAT, GR and POD under salt stress (Table 2), indicating that H$_2$S may enhance the ability of cucumber roots to scavenge ROS so that the plant can maintain membrane integrity under salt stress. The similar result was reported by Wang et al. (2012), who found that NaHS pretreatment significantly activated the antioxidant enzymes activities including SOD, CAT, POD, and APX and their expression at the transcripts level under 100 mM NaCl stress in Medicago sativa L., thus resulting in the alleviation of oxidative damage induced by NaCl.

CONCLUSION

In conclusion, exogenous application of H$_2$S alleviates the NaCl-induced toxicity via improving the growth and photosynthetic
parameters in cucumber, which could be attributed to three mechanisms: (1) \( \text{H}_2\text{S} \) increases endogenous \( \text{H}_2\text{S} \) levels via increasing the activities of L-CD and D-CD and decreasing the activities of CAS and OAS-TL in leaves or roots; (2) \( \text{H}_2\text{S} \) maintains the \( \text{Na}^+ \) and \( \text{K}^+ \) balance through regulating the expression of \( \text{PM } \text{H}^+\text{-ATPase}, \text{SOS1} \) and \( \text{SKOR} \); and (3) \( \text{H}_2\text{S} \) enhances the accumulation of antioxidants and the activities of antioxidant enzymes, leading to decline ROS accumulation and membrane lipid peroxidation. As summarized in Figure 7, \( \text{H}_2\text{S} \) alleviates salt stress in cucumber through cross talk among the mechanisms that maintain \( \text{Na}^+/\text{K}^+ \) homeostasis and regulating \( \text{H}_2\text{S} \) metabolism and oxidative stress response. In addition, the roots and leaves of cucumber showed different responses to exogenous \( \text{H}_2\text{S} \) treatment. Taken together, suitable concentration \( \text{H}_2\text{S} \) could be used to alleviate NaCl-stress induced toxicity in cucumber seedlings.

AUTHOR CONTRIBUTIONS

J-LJ designed the experiment and wrote the manuscript. YT and X-MR conducted the experiment. MY and R-PH helped in data analysis and presentation. LL revised the manuscript. All authors listed have made a substantial, direct and intellectual contribution to the work.

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Funding

This research was supported by the National Natural Science Foundation of China (31660153), Shaanxi Province Key Research and Development Project (2018NY-042), China, and Key Research Project of Shaanxi Provincial Department of Education (18JJS017), China.

Acknowledgments

We thank Anita K. Snyder, M.Sc. for the helpful suggestions and language polishing on this manuscript.

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.00678/full#supplementary-material
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.