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Research article

Hydrogen sulfide enhances rice tolerance to nickel through the prevention of chloroplast damage and the improvement of nitrogen metabolism under excessive nickel

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ABSTRACT

Hydrogen sulfide (H_2S) modulates plant tolerance to abiotic stresses, but its regulatory effects on nitrogen metabolism and chloroplast protection under nickel (Ni) stress in crop plants remain elusive. Taking this into account, we investigated the potential roles of sodium hydrosulfide (NaHS), a H_2S generator, in the improvement of growth performance of rice plants under Ni stress. Results showed that NaHS successfully reversed the adverse effects of Ni, as reflected in plant growth and biomass, and photosynthesis attributes including photosynthetic rates, stomatal conductance, transpiration rate, internal CO_2 concentration and photosynthetic pigment contents. NaHS generated H_2S plays a crucial role in controlling the photosynthetic machinery of rice as evidenced by the ultrastructure of chloroplast viewed under transmission electron microscope (TEM). The reduced content of Ni in roots and leaves of NaHS-supplemented Ni-stressed plants has revealed the restricted uptake and accumulation of Ni. A rescue of NaHS to the Ni-induced decline in nitrate (NO_3^-) content and the activities NO_3^- biosynthesizing enzymes nitrate reductase, nitrite reductase, glutamate synthase, glutamate oxaloacetate transaminase, glutamine synthetase, and glutamate pyruvate transaminase in leaves indicated a positive role of H_2S on NO_3^- metabolism in rice under Ni stress. NaHS application also reverted Ni-mediated increases in ammonium (NH_4^+) content and glutamate dehydrogenase activity, implying H_2S-induced alleviation of NH_4^+ toxicity. The regulatory effects of H_2S on nitrogen metabolism was further confirmed by increased and decreased transcript abundance of NO_3^- and NH_4^+ metabolism associated genes, respectively. Our study suggests a decisive role of H_2S in controlling Ni toxicity as elucidated by the novel findings such as enhanced gas exchanged parameters, Ni homeostasis and chloroplast protection. Moreover, this article highlights the significance of H_2S in controlling chloroplast biogenesis and nitrogen metabolism in rice crop under Ni stress.

1. Introduction

Nowadays, heavy metal accumulations throughout the ecosystem are major environmental problems, imposing threats to the quality of lives of all living organisms. Nickel (Ni) is a potential, and long-lasted environmental hazard and occupied the 22nd place among the highly

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abundant elements in the earth's crust (de Macedo et al., 2016). Being an essential micronutrient, Ni (0.01–10 μg g^{-1} dry wt) is necessary for plant to complete their life cycle (Gratao et al., 2008; Soares et al., 2016). Ni also act as co-factor of numerous enzymes, including glyoxalases, peptide deformylases, hydrogenases, superoxide dismutases and methyl-coenzyme reductase (Chen et al., 2009). Urease holds Ni at its active site (Kutman et al., 2014), which hydrolyzes urea to produce bicarbonate and ammonia (Polacco et al., 2013). Conversely, Ni at higher dose can causes severe and highly detrimental effects in seed germination, plant yield, growth and development, oxidative stress, initiation of leaf chlorosis, wilting and necrosis, and nutrient imbalances (Fabiano et al., 2015; Shukla and Gopal, 2009).

Nitrogen (N) is one of the necessary macronutrients required for plant metabolism, thus helping healthy plant growth and development (Hassan et al., 2008). Previous studies indicated that N-deficiency negatively affected plant growth by inhibiting root length and shoot branching, and decreasing soluble protein content and photosynthetic activity (Hirel et al., 2007; Lawlor, 2002). Ammonium (NH_4^+) and nitrate (NO_3^-) are the common inorganic forms of N that account for most available forms of N absorption in plants. Plants can exploit both of these ions, but their biochemical and molecular features differ from each other during their metabolisms (Luo et al., 2013). NO_3^- can be taken up by plant and converted to NH_4^+ ions that incorporate in the structure of organic molecules by nitrite reductase (NiR) and nitrate reductase (NR), the key enzymes involved in NO_3^- metabolism (Erdal and Turk, 2016; Mishra and Dubey, 2011). Commonly, the activity of NiR is greater than that of NR in plants that permits plants to evade nitrite buildup to a toxic level. However, nitrate assimilation is determined by the NR (Zhang et al., 2011). NH_4^+ can also be assimilated to glutamine (Gln) and glutamate (Glu) via glutamine synthetase (GS) and glutamate synthase (GOGAT). Excessive Ni in the growth medium may imbalance the distribution and uptake of macro- and micro nutrients, including N, due to its direct and/or indirect impact on N metabolism (Gajewska et al., 2009; Saad et al., 2016). Mishra and Dubey (2011) reported that toxic level of Ni impaired N assimilation in rice (*Oryza sativa*) seedlings by preventing the activities of NR and GS. Thus, a proper balance in N metabolism in the presence of toxic levels of Ni is vital to overcoming Ni-induced N-imbalance in economically important rice.

Plants are well equipped with several defense mechanisms in order to alleviate metal toxicity induced adverse effects. For instance, immobilization of metals in the roots, vascular compartmentalization, production of phytochelatins (PCs) and stimulation of antioxidant enzyme systems are among the crucial mechanisms (Erdal and Turk, 2016). Several growth regulators and exogenous chemicals are known to greatly contribute to the enhancement of the defense mechanisms to improve plant survival under stress conditions. Traditionally, hydrogen sulfide (H_2S) has been known as a phytotoxin, while it has appeared as a key gasotransmitter together with carbon dioxide and nitric oxide with several essential functions in plants and animals (Lisjak et al., 2013). H_2S can improve plant growth and development at low concentration, thereby contributing to the sharp increment of global food supply (Dooley et al., 2013; Savvides et al., 2016). It has already been known that H_2S influences a large number of biochemical processes, including photosynthesis in (*Hordeum vulgare*) barley and rice (Ali et al., 2013; Duan et al., 2015), root organogenesis growth in *Glycine max*, *Salix matsudana* and *Ipomoea batatas* (Zhang et al., 2009), stomata opening and closing in *Arabidopsis thaliana* (Hou et al., 2013; Lisjak et al., 2011). Additionally, induction of exogenous H_2S increased plant tolerance to numerous abiotic stresses, including drought stress in *Vicia faba*, *Arabidopsis thaliana* (García-Mata and Lamattina, 2010; Jin et al., 2011), heat stress in *Fragaria* × *ananassa* cv. Camarosa and *Zea mays* (Christou et al., 2014; Li et al., 2014b), hypoxia in *Pisum sativum* (Cheng et al., 2013), salt stress in alfalfa and *Arabidopsis thaliana* (Li et al., 2014a; Wang et al., 2012), cadmium stress in rice (Mostofa et al., 2015b), arsenic stress in pea (Singh et al., 2015) and aluminum toxicity in barley and wheat (Chen et al., 2013; Zhang et al., 2010).

Despite of having positive roles in the improvement of plant performance under abiotic stress conditions, only few studies have been conducted for evaluating the functions of H_2S in plant systems in comparison to animals. Moreover, the mechanistic insight into the regulatory roles of H_2S in plant adaptability against heavy metal stresses is limited, particularly in a main field crop like rice. Additionally, no information is available on the relationship of H_2S with Ni and N metabolism under Ni stress in plants. Considering this the current study was taken in to investigate the effects of these components on plant growth and development, Ni homeostasis, leaf gas exchange parameters, chloroplast structure, NO_3^- and NH_4^+ metabolism, activities and expression profiles of several N metabolizing enzymes in the presence and absence of NaHS in rice exposed to excessive Ni. According to our best knowledge, this study is the first of its kind to demosntrate the positive effect of H_2S on N metabolism and chloroplast ultrastructural protection in rice under Ni stress.

2. Materials and methods

2.1. Plant material, growth environment and treatments

Healthy rice seeds (*Oryza sativa* L., cv. yangliangyou 6) were surface sterilized with 10% (v/v) H_2O_2 for 10 min followed by washing 6 times with distilled water before imbibition in the dark for 24 h. For germination, seeds were kept on plastic nets floating on distilled water in plastic pots and kept in dark at 28 ± 2 °C for 96 h. Uniformly germinated seeds were transferred to 4 L plastic box containing half-strength nutrient solution and grown in a controlled room (28 ± 1 °C, 80% relative air humidity with light intensity of 820 mmol m^{-2} s^{-1} 16 h light/8 h dark cycle). The nutrient solution contained the following elements (in mg/L): 40 N, 10 P, 40 K, 40 Ca, 40 Mg, 0.5 Mn, 0.05 Mo, 0.2 B, 0.01 Zn, 0.01 Cu, 2 Fe, which were added in the form of NH_4NO_3, $NaHPO_4{\cdot}2H_2O$, K_2SO_4, $CaCl_2$, $MgSO_4{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$, $(NH_4)_6Mo_7O_4{\cdot}4H_2O$, H_3BO_3, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $FeCl_3.6H_2O$, and citric acid (monohydrate), respectively (Yoshida et al., 1976). Twenty one-days-old rice plants were exposed to a nutrient solution containing Ni as $NiSO_4.6H_20$ and H_2S as NaHS. Four different treatments of Ni and NaHS were ensured in this experiment including (1) control (CK), (2) 100 μM NaHS, (3) 200 μM Ni, (4) 100 μM NaHS + 200 μM Ni. These concentrations were selected on the basis of previous study (Rizwan et al., 2017). Based on literature (Li et al., 2012; Mostofa et al., 2015b), and our early experiments with a range of NaHS concentrations (50, 100, 150 and 200 μM), we observed that 100 μM NaHS effectively alleviated the Ni-induced toxic symptoms in rice. Additionally, we tested a series of chemicals such as Na_2S, Na_2SO_3, Na_2SO_4, $NaHSO_3$ and $NaHSO_4$ (100 μM) as NaHS homologues in order to confirm whether NaHS was the actual donor of H_2S. For further confirmation of the roles of H_2S in the enhancement of Ni-tolerance in rice plants, hypotaurine (200 μM) (Lai et al., 2014; Mostofa et al., 2015b) and potassium pyruvate (PP, 200 μM) were used as H_2S scavenger and H_2S biosynthetic inhibitor, respectively. The solutions of these treatments were renewed every three days and the pH was adjusted to 6.0 using HCl or NaOH. At day 14th of treatment with Ni, rice plants were harvested to examine H_2S-induced mechanisms controlling biochemical, physiological, ultrastructural and molecular responses. Every treatment were repeated three times by maintaining the same environmental conditions.

2.2. Determination of plant growth

To determine the plant height, the length of the main stem from its bottom to newly emerged leaf was measured using a meter scale. For the determination of biomass of rice plants, fresh weight (FW) and dry weight (DW) of 10 seedlings from each treatment were weighed before and after oven drying at 65 °C for 72 h respectively. The data was

articulated as g seedlings^{-1}.

2.3. Determination of Ni content

To measure the Ni concentration, the shoot, and root samples were separately collected and oven dried at 65 °C for 72 h. Oven dried (0.1 g) samples were processed at 140 °C by using a mixture of concentrated acid solutions (HNO_3: $HClO_4$ at 4:1) till the disappearance of the yellow color. The Ni contents in the digested solutions were determined using Atomic Absorption Spectrophotometer (AAS: Agilent Technologies, 200 series AA).

2.4. Determination of H_2S content

H_2S content in the leaves of rice was measured according to Christou et al. (2013) with slight modification as suggested by Mostofa et al. (2015a). Leaf samples (0.25 g) were crushed with mortar and pestle in 1.0 ml of potassium phosphate buffer (100 mM, pH 7.0), containing EDTA (10 mM) and followed by centrifugation at 11,200 × *g* for 15 min. The resulting supernatant (100 μL) was mixed with extraction buffer (1880 μL) and 20 mM 5,5′ -dithiobis (2-nitrobenzoic acid) (20 μL) and was incubated for 5 min at 25 °C. The absorbance was recorded at 412 nm, and the content of H_2S content was calculated with the help of a standard curve obtained from the known concentrations of NaHS.

2.5. Gas exchange measurements and photosynthetic pigments

The gas exchange measurements, such as net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and intercellular CO_2 concentration (Ci) were monitored by using portable infrared gas analyzer ((LI-6400XT, LI-COR Inc., Lincoln, NE, USA). All the measurements were recorded in the time interval of 9:00 to 12:00 a.m. For the determination of the contents of photosynthetic pigments chlorophyll and carotenoids, rice leaves (0.5 g) were extracted using 80% chilled acetone followed by recording of the resultant supernatants at at 470 nm, 645 nm, and 663 nm by using a spectrophotometer (Beckman 640 D, USA). Chlorophyll (Chl) and carotenoids contents were determined according to the formulae suggested by Lichtenthaler and Wellburn (1983) and expressed as mg g^{-1} FW.

2.6. Transmission electron microscopy

Fresh leaf samples without veins were taken randomly and fixed in 4% glutaraldehyde (v/v) in 0.2 M sodium phosphate buffer (pH 6.8) for 6 h at 4 °C followed by washing with the same buffer. Samples were postfixed in 1% osmic acid in 0.2 M PBS (pH 6.8) for 2.5 h, cleaned three times in 0.2 M PBS (pH 6.8). Subsequently with the break of 15–20 min, the samples were parched in a sorted series of ethanol (50–100%) and cleaned by absolute acetone for 20 min. After this permeation, embedding and slicing ultra-thin sections was carried out according to the method reported in Meng et al. (2014). The samples were examined under a transmission electron microscope (H-7650; Hitachi, Japan) at 100 kV. The numbers of deformed and normal chloroplasts were calculated using 10 micrographs per case.

2.7. Determination of NO_3^- and NH_4^+ contents

NO_3^- and NH_4^+ contents in freshly harvested rice leaves were determined using the kits (ZXTD-2-G for NO_3^--N and ZATD-2-G for NH_4^+-N) purchased from Comin Biotechnology Co. Ltd, China (http://www.cominbio.com). Guidelines given by the manufacturing company about the use of kits were strictly followed, and the absorbance was read at 410 and 580 for NO_3^- and NH_4^+, respectively, and expressed the units in μg g^{-1} FW for both parameters.

2.8. Determination of N metabolism-related enzyme activities

The activities of N metabolizing enzymes NR, NiR, GS, GOGAT, GDH, GOT and GPT were determined in the fresh leaves of rice plants under investigations. The activities of NR and NiR were determined using the Kits NR-2-Y and NIR-2-G, respectively. One unit (U) of enzyme activity was calculated as the number of enzymes required to yield 1 μM NO_2^- per h per mg of protein for both enzymes. The GS activity was assessed using GS detection Kit (GS-2-Y), and 1 U of enzyme activity was defined as per milligram of protein in per mL reaction system to make the absorption to change by 0.01 per min at 540 nm. The GOGAT activity was monitored using GOGAT detection Kit (GOGAT-2-Y), and 1 U enzyme activity was defined as per milligram protein to consume 1 nmol NADH per min. Assay of GDH activity was carried out by using GDH-2-Y kit and 1 U enzyme activity was defined as per milligram of tissue protein in the reaction system to make the absorption to change by 0.01 per min. The Glutamate pyruvate transaminase (GPT) and Glutamate oxaloacetate transaminase (GOT) activities were tested by using the kits (GOT-2-Y for GOT and GPT-2-Y for GPT). One U of enzyme activity was defined as per g of protein in the reaction system to make the absorption to change by 0.01 per min. All the kits for analyzing enzyme activities were purchased from Comin Biotechnology Co. Ltd, China (http://www.cominbio.com).

2.9. Total RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA from leaves of rice seedlings was extracted using RNA kit (Biotech, Beijing) in line with the instructions of the manufacturer. To remove the genomic DNA contamination, 10 μg of total RNA were digested with RNase-free DNaseI (Promega, Madison, WI). RNA concentration was measured with the help of a NanoDrop, i.e. ND-2000 UV spectrophotometer (Thermo Scientific, USA) after DNaseI treatment. First-strand cDNA was produced from 2 μg total RNA with the help of Superscript III first strand synthesis system (Invitrogen). Entire cDNA samples were diluted 50-fold with sterile water to obtain qRT-PCR. The particular primer of individual gene was applied (Provided in Supplementary Table S1). Rice ACTIN was utilized as inner control. qRT-PCR calculations were executed in 96-well plates (iQ5 Real Time PCR System; Bio-Rad) for all treatments with 3 biological replication and every replication distributed into 2 technical replication in a CFX-96 Bio-Rad thermocycler (Bio-Rad). The qRT-PCR was accomplished with the conditions: denature the DNA at 94 °C for 3 min, followed by 40 cycles of 94 °C (20 s), 58 °C (20 s), and 72 °C (20 s). The relative variance in expression was calculated by normalizing the Ct value for every gene compared to the Ct value of ACTIN for each sample. It was considered relative to the particular control samples as calibrator using Equation (2)$^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001).

2.10. Statistical analysis

The data were analyzed using Tukey's test (HSD) and one-way analysis of variance (ANOVA) with statistical package Statistix 8.1. Data were represented as means ± standard deviations (SD) for each treatment.

3. Results

3.1. H_2S, among other derivatives, contributed to the alleviation of rice seedlings under Ni stress

To differentiate the role of H_2S to other sodium or sulfur containing derivatives in the increase of Ni-induced stress, we used number of sodium and sulfur containing compounds, such as Na_2S, Na_2SO_3, Na_2SO_4, $NaHSO_3$ and $NaHSO_4$. As shown in Fig. 1, plant height, FW, DW and H_2S content in rice seedling under Ni and NaHS treatment were

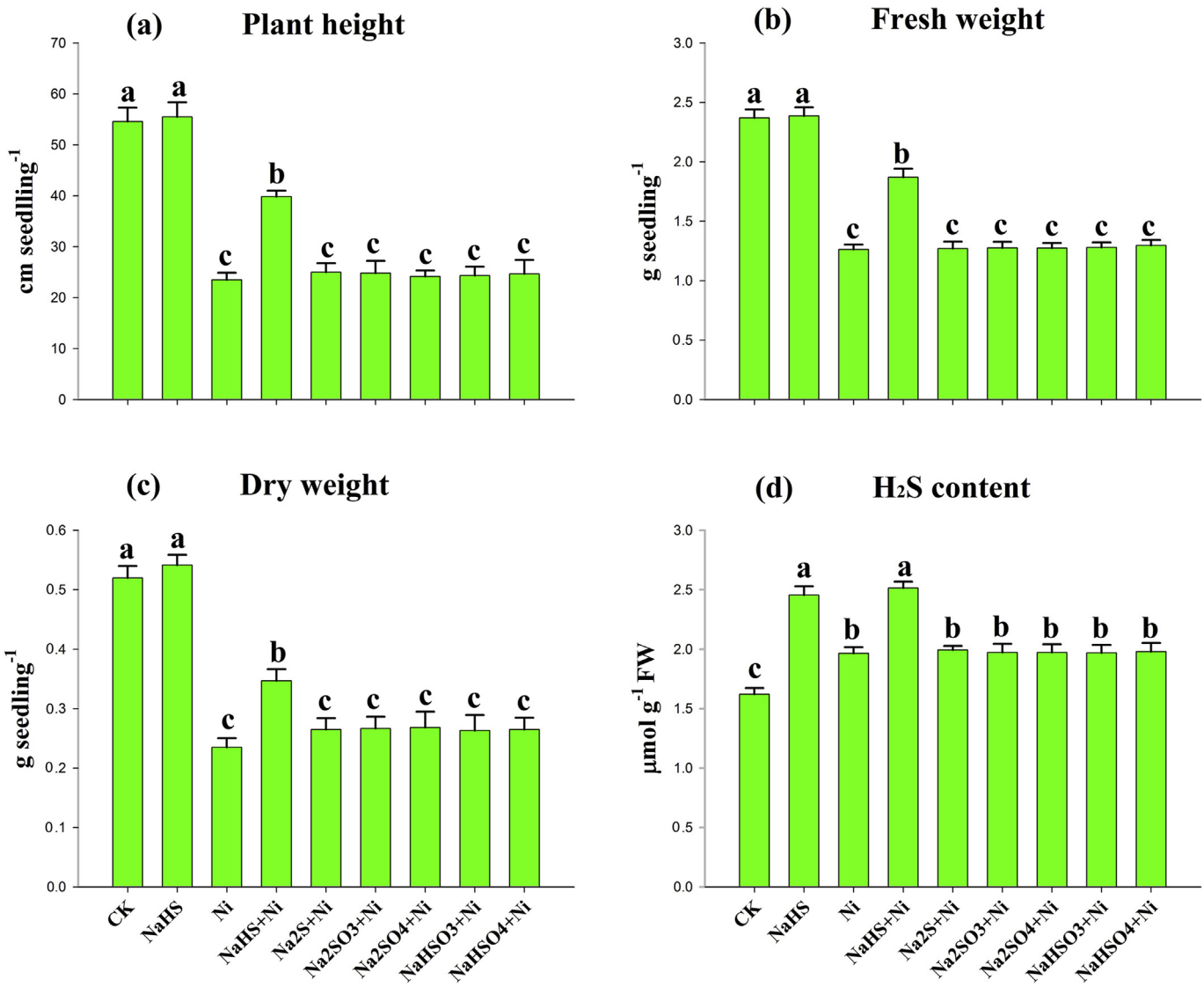

Fig. 1. Role of various exogenous materials on plant height **(a)**, FW **(b)**, DW **(c)** and hydrogen sulfide (H_2S) content **(d)** in rice seedling of treatment with control (CK, only Hoagland nutrient solution), 100 µM NaHS (NaHS), 200 µM Ni (Ni), 100 µM NaHS + 200 µM Ni (NaHS + Ni), 100 µM Na_2S + 200 µM Ni (Na_2S + Ni), 100 µM Na_2SO_3 + 200 µM Ni (Na_2SO_3 + Ni), 100 µM Na_2SO_4 + 200 µM Ni (Na_2SO + Ni), 100 µM $NaHSO_3$ + 200 µM Ni ($NaHSO_3$ + Ni), 100 µM $NaHSO_4$ + 200 µM Ni ($NaHSO_4$ + Ni). Bars shows means ± standard deviations (SDs) of three independent replications ($n = 3$). Means followed by the same letter are non-significant among the treatments at $P < 0.05$ considering Tukey's (HSD) test. FW; fresh weight, DW; dry weight.

higher, than those of Ni-treated only plants. Conversely, no significant improvement in the growth parameters and H_2S content was detected after the application of Na_2S, Na_2SO_3, Na_2SO_4, $NaHSO_3$ and $NaHSO_4$ to Ni-stressed seedlings (Fig. 1). These results suggest that NaHS dissociated H_2S contributed to the enhancement of Ni tolerance in rice seedlings.

3.2. Effect of H_2S scavenger and its biosynthesis inhibitor on H_2S-induced Ni tolerance in rice seedlings

To be more precise about whether the increasing effect of NaHS was tangled in H_2S during rice growth under Ni toxicity. H_2S scavenger HT (200 µM) and H_2S biosynthesis inhibitor PP (200 µM), and combination of PP with NaHS was exposed to Ni toxicity. As shown in Fig. 2, addition of NaHS to Ni-toxic seedlings increased the plant growth in comparison to only Ni-stressed seedlings. Furthermore, the defensive response of NaHS on the amelioration of Ni-induced decrease in growth was stopped on the application of HT and PP. In comparison with control, an enhancement of H_2S content was observed in Ni-treated plants. As anticipated, increase in H_2S content was calculated in rice seedlings under NaHS treatment. Conversely, no significant rise of H_2S content was detected on the application of HT and PP in rice leaves (Fig. 2). These results indicated that H_2S released from NaHS participate in Ni tolerance mechanism of rice plants subjected to Ni stress.

3.3. H_2S suppresses Ni uptake to overcome Ni toxicity

To investigate whether the beneficial effect of exogenous NaHS application on Ni-stressed seedlings was related to its capability to suppress Ni uptake, Ni content in the roots and leaves were determined with or without application of NaHS. Ni content in the shoots and roots of Ni-stressed seedlings sharply increased (Fig. 3a and b). However, the Ni content decline to 38% in roots and 10% in shoots, in the NaHS + Ni applied seedlings in comparison to alone treatment of Ni-stressed seedlings (Fig. 3a and b).

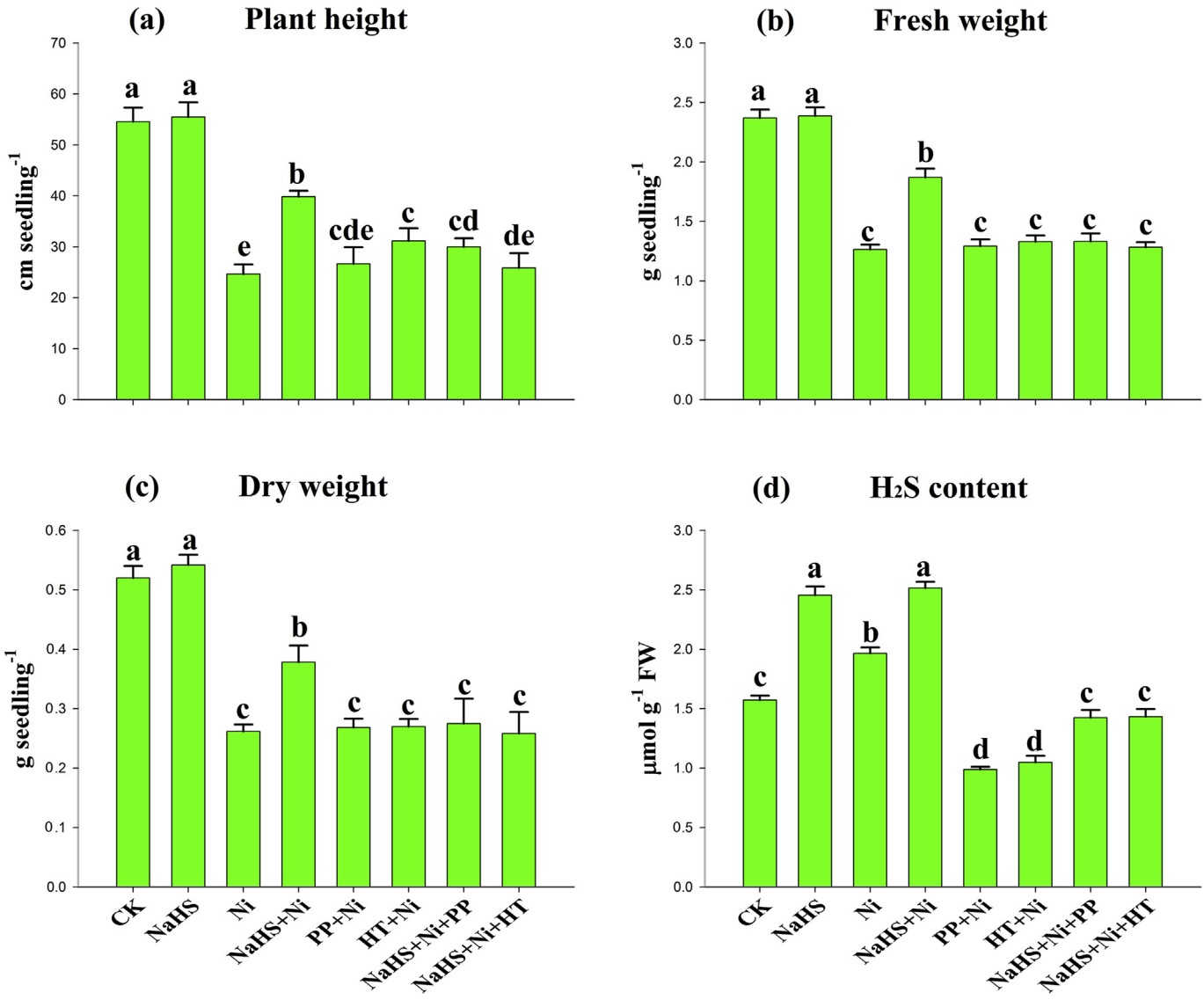

Fig. 2. Role of hydrogen sulfide (H_2S) synthesis inhibitor potassium pyruvate (PP, 200 µM) and H_2S scavenger hypotaurine (HT, 200 µM) on plant height **(a)**, FW **(b)**, DW **(c)** and H_2S content **(d)** in old rice seedling of treatment with control (CK, only Hoagland nutrient solution), 100 µM NaHS (NaHS), 200 µM Ni (Ni), 100 µM NaHS + 200 µM Ni (NaHS + Ni), 200 µM PP + 200 µM Ni (PP + Ni), 200 µM HT + 200 µM Ni (HT + Ni), 100 µM NaHS + 200 µM Ni + 200 µM PP (NaHS + Ni + PP) and 100 µM NaHS + 200 µM Ni + 200 µM HT (NaHS + Ni + HT). Bars shows means ± standard deviations (SDs) of three independent replications ($n = 3$). Means followed by the same letter are non-significant among the treatments at $P < 0.05$ considering Tukey's (HSD) test. FW; fresh weight, DW; dry weight.

3.4. H_2S improves plant growth under Ni stress

Exposure of rice seedlings to Ni exhibited clear toxicity symptoms, as observed by growth retardation, leaf rolling and chlorosis in Ni-challenged rice seedlings (Fig. 3c). However, these toxicity symptoms were almost diminished by application of exogenous NaHS (Fig. 3c). As expected, plant height, FW and DW of the Ni-treated seedlings reduced by 36%, 40% and 56%, as compared with control. However, application of NaHS with Ni minimized the harmful effects on plant height, FW and DW, and it was recovered by 31%, 29% and 63% respectively, as compared with Ni-treated only seedlings. These results showed that addition of exogenous addition of NaHS could increase Ni-tolerance of rice under Ni stress (Table 1).

3.5. H_2S maintains gas exchange parameters in response to Ni stress

Ni toxicity caused a sharp decline in Pn, Ci, Tr and GS in leaves by 45%, 43%, 74% and 78% of the control (Table 1). The negative impacts of Ni toxicity on gas exchange parameters were significantly overcome by exogenous NaHS, which amplified the values of Pn, Ci, Tr and Gs by 61%, 62%, 233% and 274%, respectively, relative to Ni-stressed only seedlings (Table 1).

3.6. H_2S protects photosynthetic pigments

Ni stress caused deleterious effects on rice leaves, resulting chlorosis and necrosis along rice leaf margin (Fig. 3c). However, combined application of Ni and NaHS have almost nullified these toxic symptoms on rice leaves (Fig. 3c). Table 2 highlights that, the Ni stress induced a significant decline in the contents of Chl a, Chl b, Chl a+b and carotenoids, which were decreased by 21%, 20%, 21% and 10%, respectively, with respect to the control. In contrast, the levels of Chl a, Chl b, Chl a+b and carotenoids in rice leaves significantly increased the addition of exogenous NaHS under Ni stress conditions, resulting in increases by 22%, 21%, 22% and 6%, respectively compared to Ni-stressed seedlings (Table 2).

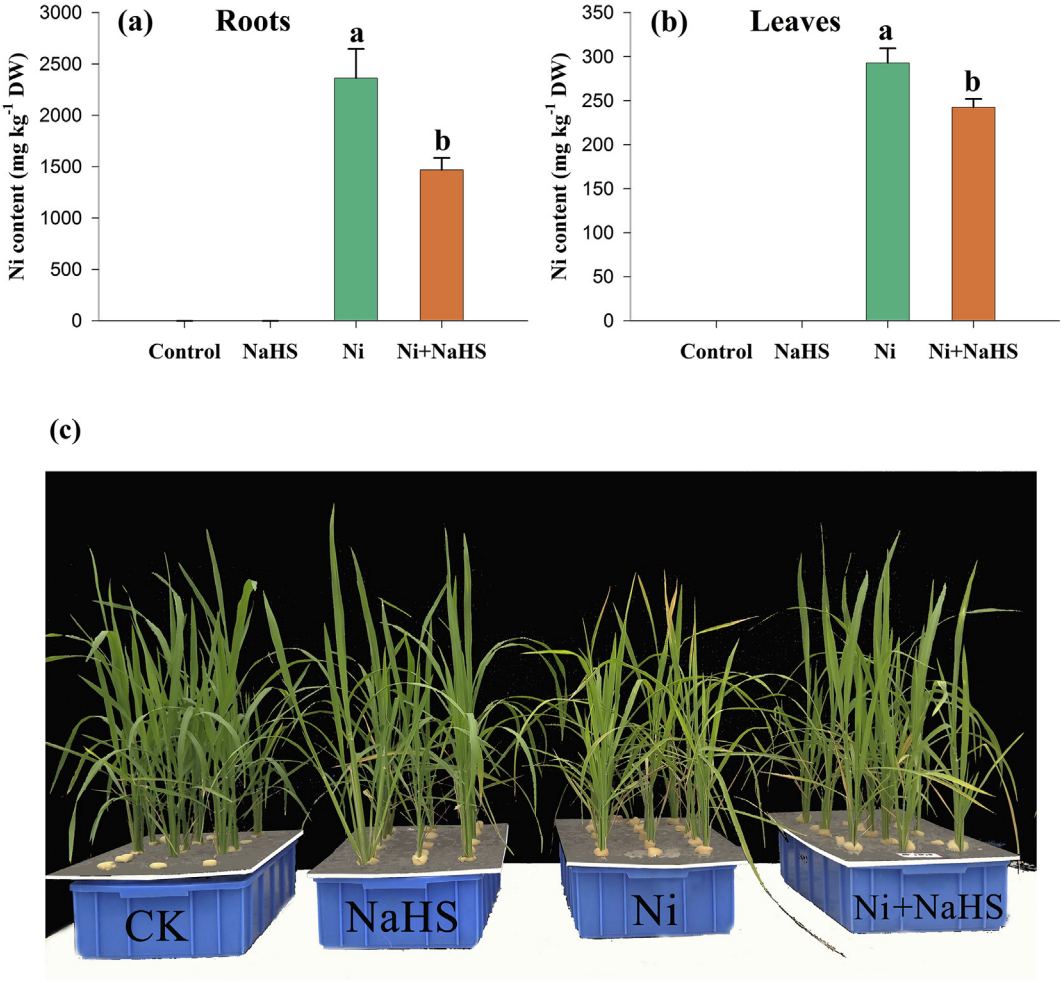

Fig. 3. Role of NaHS on the contents of Ni in roots **(a),** leaves **(b)** and on the phenotype **(c)** of rice plants subjected to Ni stress for a period of 2 weeks. Control, NaHS, Ni, Ni + NaHS represents the group of plants treated with nutrient solutions containing nutrients only, 100 μM sodium hydrosulfide, 200 μM Ni, and 100 μM sodium hydrosulfide +200 μM Ni, respectively. Bars shows means ± standard deviations (SDs) of three independent replications ($n = 3$). Means followed by the same letter are non-significant among the treatments at $P < 0.05$ considering Tukey's (HSD) test.

3.7. H_2S protects the ultrastructure of chloroplasts from Ni-induced damage

An observable modification can be noticed in the chloroplasts of Ni-stressed seedlings, as compared with control, such as chloroplast were more swelled with destroyed thylakoid system, increases in number of plastoglobuli with less starch granules can be seen as compared with control (Fig. 4c). Whereas, NaHS in Ni combination reverted the toxic effect of Ni such as, regular chloroplast structure with all the parts like thylakoid, grana and starch granules, but plastoglobuli were decreased as compared to Ni-stressed seedlings only (Fig. 4d).

3.8. H_2S balances NO_3^- and NH_4^+ contents in rice leaves

Fig. 5 depicts that Ni-stressed reduced the NO_3^- content in rice leaves by 24% as compared with the control. Exogenous NaHS supplementation significantly elevated the NO_3^- content in the Ni-stressed seedlings (Fig. 5a). On the contrary, Ni-stressed triggered the accumulation of NH_4^+ in rice leaves, which increased by 14% as compared with control. However, NaHS application to rice seedlings in Ni-stressed conditions further blocked the accumulation of NH_4^+ content, and caused a decrease of NH_4^+ content by 34% as compared with Ni-stressed seedling only (Fig. 5b).

Table 1
Role of NaHS on growth-related and gas exchange mechanisms of rice plants exposed to Ni stress for a period of 2 weeks. Control, NaHS, Ni, Ni + NaHS represents the group of plants treated with nutrient solutions containing nutrients only, 100 μM sodium hydrosulfide, 200 μM Ni, and 100 μM sodium hydrosulfide +200 μM Ni, respectively.

Treatments	Plant height (cm^{-1} seedling)	FW (g^{-1} seedling)	DW (g^{-1} seedling)	Pn ($\mu mol.m^{-2}.s^{-1}$)	Gs ($mol.m^{-2}.s^{-1}$)	Tr ($mmol.m^{-2}.s^{-1}$)	Ci ($\mu mol.mol^{-1}$)
Control	49.46 ± 2.01b	2.49 ± 0.06b	0.55 ± 0.02a	9.07 ± 0.64b	0.19 ± 0.05a	5.35 ± 1.15a	290.56 ± 18.20a
NaHS	52.51 ± 1.38a	2.58 ± 0.04a	0.58 ± 0.02a	10.81 ± 0.54a	0.24 ± 0.09a	5.82 ± 1.68a	278.47 ± 39.82a
Ni	31.51 ± 1.38d	1.48 ± 0.05d	0.24 ± 0.02c	4.96 ± 0.79d	0.04 ± 0.01b	1.41 ± 0.23b	164.54 ± 33.64b
Ni + NaHS	41.27 ± 1.36c	1.90 ± 0.03c	0.39 ± 0.02b	7.99 ± 0.52c	0.16 ± 0.06a	4.69 ± 1.63a	266.91 ± 40.36a

Values are means ± standard deviations ($n = 6$). Means followed by the same letter are non-significant among the treatments within the same column at $P \leq 0.05$ considering Tukey's (HSD) test. FW; fresh weight, DW; dry weight, Pn, net photosynthesis rate; Gs, stomatal conductance; Tr, transpiration rate and Ci, intercellular CO_2 concentration.

Table 2
Role of NaHS on the contents of photosynthetic pigments in the leaves of rice plants treated with Ni stress for a period of 2 weeks. Control, NaHS, Ni, Ni + NaHS represent the group of plants treated with nutrient solutions containing nutrients only, 100 μM sodium hydrosulfide, 200 μM Ni, and 100 μM sodium hydrosulfide +200 μM Ni, respectively.

Treatments	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Control	2.08 ± 0.05b	0.73 ± 0.13 ab	2.82 ± 0.17b	0.41 ± 0.02b
NaHS	2.28 ± 0.03a	0.84 ± 0.02a	3.12 ± 0.05a	0.45 ± 0.02a
Ni	1.66 ± 0.06c	0.58 ± 0.02b	2.24 ± 0.08c	0.37 ± 0.01c
Ni + NaHS	2.02 ± 0.04b	0.69 ± 0.04 ab	2.73 ± 0.05b	0.39 ± 0.02b

Values are means ± standard deviations ($n = 3$). Means followed by the same letter are non-significant among the treatments within the same column at $P \leq 0.05$ considering Tukey's (HSD) test. FW; fresh weight.r

3.9. H_2S boosts the activities of N metabolism related enzymes

Ni-stressed seedlings showed a drastic decline in the activities of NR and NiR by 17 and 31%, in comparison with control. However, NaHS application improved the activities of both NR and NiR in the rice leaves under Ni stress by 12% and 33% over those of Ni-stressed seedlings only (Fig. 5c and d). In comparison with control, the GS and GOGAT activities significantly decreased by 39% and 44% in rice leaves under Ni toxicity (Fig. 5e and f). In contrast, GDH activity significantly increased by 40% under Ni-stressed seedlings, when compared with control (Fig. 5g). Exogenous NaHS treatment caused an enhancement in the GS, GOGAT activities, by 23% and 42%, respectively, while GDH activity was suppressed by 40%, as compared to Ni-stressed seedlings (Fig. 5e–g). The activities of GOT and GPT were significantly inhibited under Ni stress by 45% and 21% in rice leaves, respectively, as compared to control (Fig. 5h and i). However, NaHS in Ni combination further blocked the enhancement of GOT and GPT activities than Ni-challenged only seedlings. Moreover, NaHS application alone enhanced the activities of GOT and GPT as compared to control (Fig. 5h and i).

3.10. Transcriptional regulation of genes involved in N uptake and metabolism

Because rice seedling disclosed morphological and physiological changes in responses to Ni stress and NaHS application, internal molecular responses may also be predictable in the transcriptional regulation pattern of key genes implicated in N uptake and metabolism. We used qRT-PCR to analyze the expression of *OsNRT1;1, OsAMT1;1, OsNR, OsNiR, OsGS2, OsFd-GOGAT, OsNADH-GOGAT* and *OsGDH1*. The transcript levels of *OsNRT1;1* and *OsAMT1;1* in leaves of rice were clearly affected by Ni, and NaHS + Ni treatments. Under Ni stress, the transcriptional expression of nitrate transporter family member *OsNRT1;1* significantly decreased, while increasing the transcriptional expression of ammonium transporter family member *OsAMT1;1*. Exogenous NaHS application up-regulated *OsNRT1;1* and down-regulated *OsAMT1;1* expression level in rice leaves compared to that only Ni-stressed leaves (Fig. 6a and b). The expression levels of *OsNR, OsNiR, OsGS2, OsFd-GOGAT* and *OsNADH-GOGAT* were downregulated and caused an increase in expression level of *OsGDH1* in rice leaves under Ni-stressed only seedlings (Fig. 6c–h). However, application of NaHS significantly increased the expression levels of *OsNR, OsNiR, OsGS2, OsFd-GOGAT,* and *OsNADH-GOGAT,* whereas decreased the expression level of *OsGDH1* in rice leaves, when compared with Ni-stressed only seedlings (Fig. 6c–h).

4. Discussion

Growth inhibition, biomass reduction, impaired water balance and decline in photosynthetic activity are among the numerous phytotoxic consequences of Ni toxicity in plants. The addition of the HT and PP, to Ni-treated seedlings blocked further production of H_2S in rice leaves. We found a low H_2S content and similar FW and DW as compared to Ni-treated seedling only. However, there is less effect on plant height after addition of HT and PP in Ni-treated Seedlings, although the increase in plant height is not so high as compared to Ni-treated seedling only (Fig. 2a–d). In this study, our results indicated that Ni contents in shoots and roots of Ni-challenged only rice seedling were sharply increased (Fig. 3a and b), when compared with Ni-free control seedlings. These

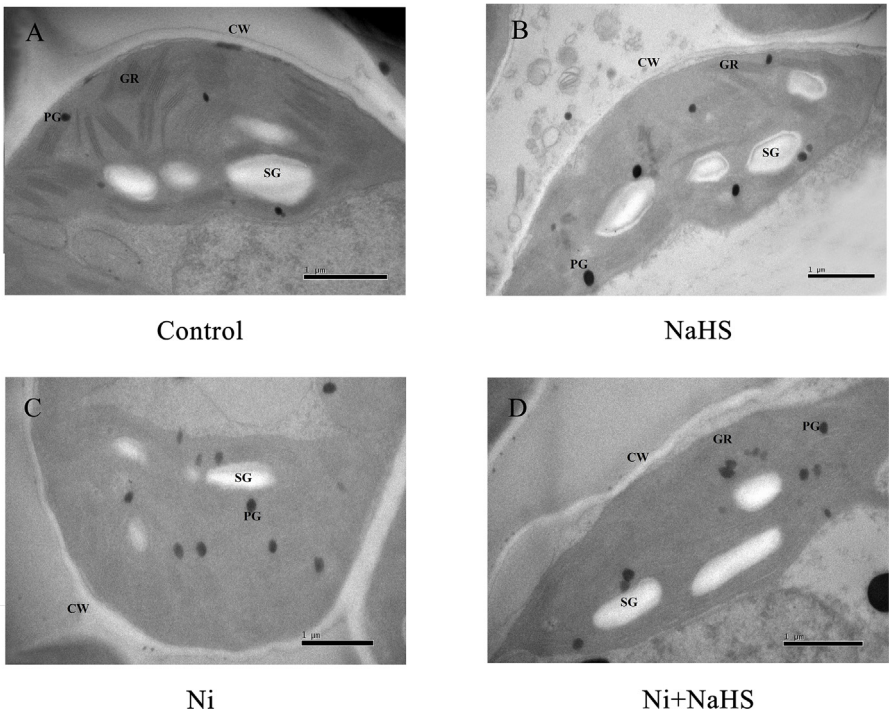

Fig. 4. Role of NaHS on Ni-induced ultrastructural changes in chloroplasts of rice leaves. Control, NaHS, Ni, Ni + NaHS represents the group of plants treated with nutrient solutions containing nutrients only, 100 μM sodium hydrosulfide, 200 μM Ni, and 100 μM sodium hydrosulfide+ 200 μM Ni, respectively. CW, cell wall; SG, starch grains; PG, plastoglobuli; GR, grana.

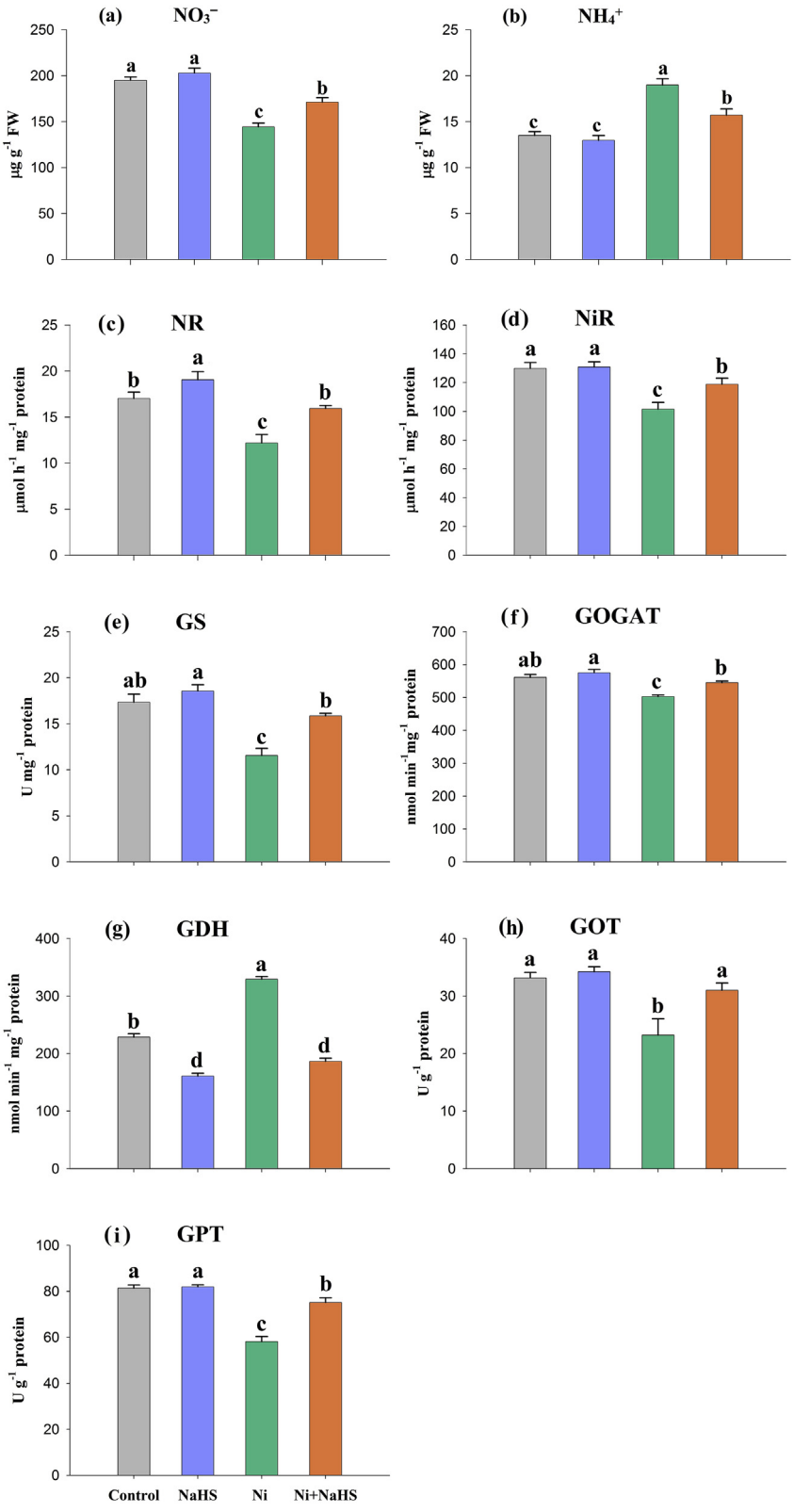

Fig. 5. Role of NaHS on the nitrate (NO_3^-) level **(a),** ammonium (NH_4^+) **(b),** and activities of nitrate reductase (NR) **(c)**, nitrite reductase (NiR) **(d)**, glutamine synthetase (GS) **(e)**, glutamate synthase (GOGAT) **(f)**, and glutamate dehydrogenase (GDH) **(g),** glutamate oxaloacetate transaminase (GOT) **(h)** and glutamate pyruvate transaminase (GPT) **(i)** in leaves of rice seedling in presence and absence of Ni. Control, NaHS, Ni, Ni + NaHS represent the group of plants treated with nutrient solutions containing nutrients only, 100 µM sodium hydrosulfide, 200 µM Ni, and 100 µM sodium hydrosulfide + 200 µM Ni, respectively. Bars shows means ± standard deviations (SDs) of three independent replications ($n = 3$). Means followed by the same letter are non-significant among the treatments at $P < 0.05$ considering Tukey's (HSD) test.

findings are in line with the results observed in *Glycine max* and *Triticum aestivum* (Sirhindi et al., 2016: Siddiqui et al., 2011) under Ni stress. In relation to higher amount of Ni in shoots and roots of Ni-stressed rice plants, we found that excessive Ni severely hampered rice growth performance by reducing plant height, FW and DW (Table 1), and these findings are in agreement with the results of Rehman et al. (2016) in maize, Khaliq et al. (2016) in cotton and Sirhindi et al. (2016) in *Glycine max*. Reports suggested that Ni-mediated reduction in root growth was due to distortion of cell nucleus and nucleolus of tomato root tips (Mosa et al., 2016) and mitotic activity inhibition, which ultimately affects the overall plant growth (Gajewska et al., 2009). However, addition of NaHS (H_2S donor) significantly suppressed Ni accumulation in roots and leaves of rice plant and reverted the consequent negative effects of Ni stress on plant growth (Fig. 3a–c), as was observed in *Hordeum vulgar*

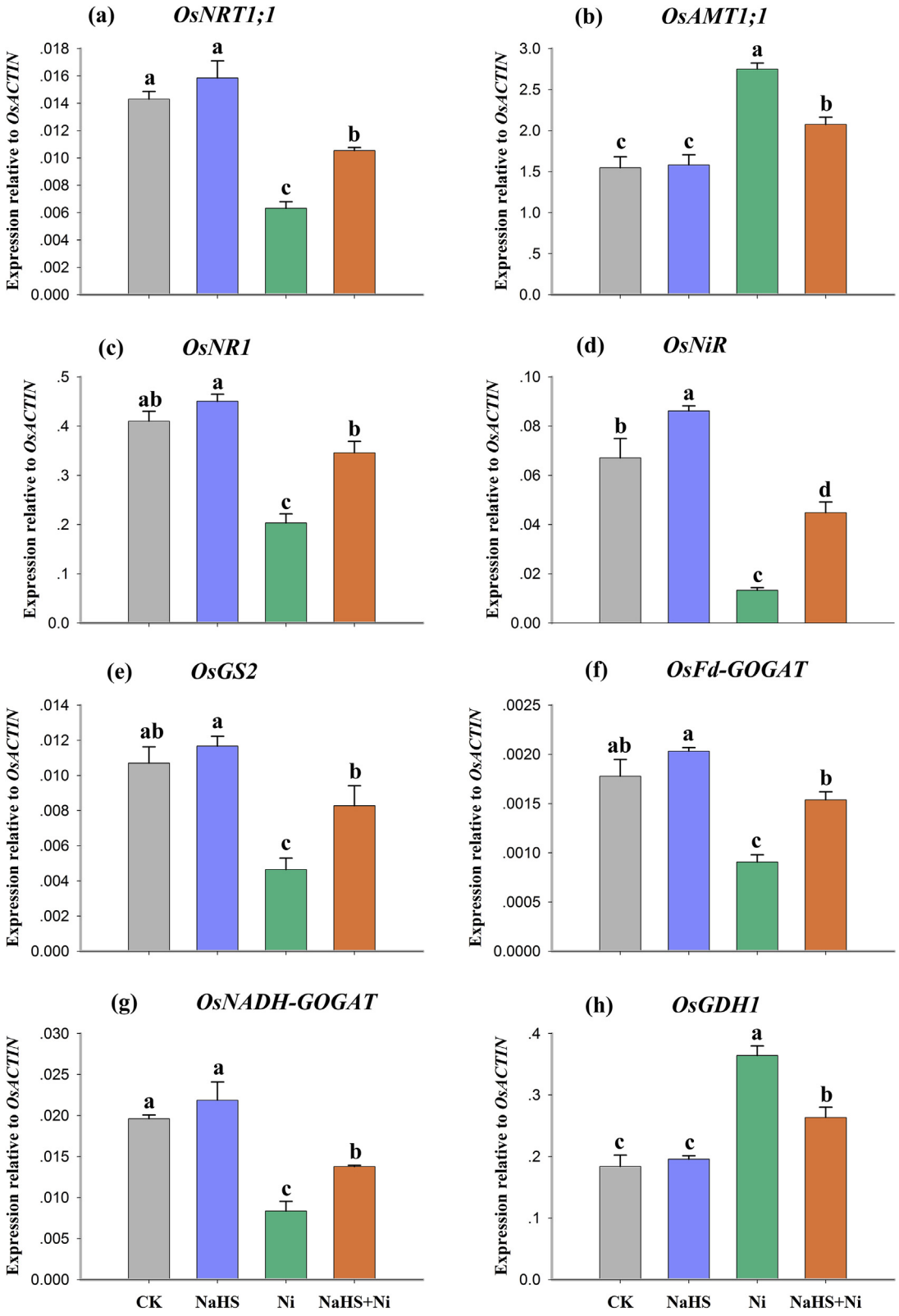

Fig. 6. Effects of NaHS on transcriptional expression of *OsNRT1;1* **(a)**, *OsAMT1;1* **(b)**, *OsNR1* **(c)**, *OsNiR* **(d)**, *OsGS2* **(e)**, *OsFd-GOGAT* **(f)**, *OsNADH-GOGAT* **(g)** and *OsGDH1* **(h)** in the leaves of rice plants in presence and absence of Ni. Control, NaHS, Ni, Ni + NaHS represent the group of plants treated with nutrient solutions containing nutrients only, 100 μM sodium hydrosulfide, 200 μM Ni, and 100 μM sodium hydrosulfide +200 μM Ni, respectively. Bars shows means ± standard deviations (SDs) of three independent replications ($n = 3$). Means followed by the same letter are non-significant among the treatments at $P < 0.05$ considering Tukey's (HSD) test.

(Dawood et al.,2012).

Chl content is a useful indicator of stress tolerance and often used as parameter of chloroplast development and photosynthetic capacity. Under environmental stress conditions, Chl contents in different plant species vary depending on the duration and the intensity of stresses (Yuan et al., 2012). In this study, Ni stress caused a drastic inhibition of photosynthetic pigments (Table 2) and the results are in accordance with the findings of Sirhindi et al. (2016) in *Glycine max* and Mosa et al. (2016) in tomato. Moreover, under Ni stress condition, Pn, Gs, Tr and Ci were decreased and the results are in agreement with the results of Rehman et al. (2016) in *Zea mays* L. In the present study, the ultrastructure of the chloroplasts was greatly altered demonstrating the distorted shapes of chloroplasts under Ni stress, as compared with control (Fig. 4c). Our findings are corroborated well with the results found in tomato leaves under Ni stress (Mosa et al., 2016). On the other hand, the negative effects of Ni stress on photosynthetic pigments, gas exchange measurements and chloroplasts ultrastructure were successfully alleviated by the addition of exogenous NaHS. These results showed that H_2S, as a signaling molecule could stimulate the biogenesis of chloroplasts by uplifting the quantity of grana lamellae and the biosynthesis of chlorophyll, which together might have boosted the photosynthetic capacity of Ni-challenged rice plants.

N is an integral part of basic N-containing compounds such as nucleic acids and proteins. Thus, N metabolism has been linked to photosynthetic carbon assimilation and performs highly significant roles during plant growth and development (Cameron and Haynes, 1986). Plants mostly uptake inorganic nitrogen as NO_3^- and NH_4^+ from soil. Plant roots absorb NO_3^-, which then converts into to NH_4^+ by the enzymes NR and NiR. In our study, we demonstrated that Ni stress significantly reduced the NO_3^- content in rice leaves (Fig. 5a). In consistence with the decrease in NO_3^- content, downregulation of nitrate transporter (*OsNRT1;1*) was found in response to Ni stress. These findings are in agreement with the results in wheat leaves (Gajewska et al., 2009) and rice leaves (Mishra and Dubey, 2011) under Ni stress. It was suggested that high Ni concentration limits NO_3^- uptake from roots, and/or its transportation to the shoots. The current study also showed inhibition in NR activity (Fig. 5c), and reduced transcript level of *OsNR1* gene in rice leaves under Ni stress. NR is a key enzyme in N metabolism, which catalyzes NO_3^- reduction to nitrite (NO_2^-) and is very sensitive to various stressors (Boussama et al., 1999). Several earlier studies reported the inhibition of NR activity when exposed to Ni stress (Gajewska et al., 2009; Mishra and Dubey, 2011). Inhibition of NR activity due to Ni stress might be due to (1), low affinity of this enzyme towards NO_3^- ions (Sharma and Dubey, 2005) (2), increase in reactive oxygen species (ROS) concentration (3) and reduced NO_3^- availability to rice shoots could also suppresses the transcript levels of *OsNR1* gene and reduced the stability of NR mRNAs. In this study, exogenous NaHS treatment significantly increased the NO_3^- content and enhanced NR activity at the transcription level of Os*NR1* under Ni stress. These results demonstrated that NO_3^- uptake and/or its transport to leaves was induced by NaHS, and finally assimilation of N was activated with the enhancement of NR activity. This finding also suggest that NaHS exerts its effect both at activity at genetic level. Similar to NR, NiR activity also showed inhibition in response to Ni stress and downregulation of the expression level of Os*NiR*. Our findings are in line with the findings of Gajewska et al. (2009), who reported a decline in NiR activity in wheat shoots under Ni toxicity. The decline in NiR activity may be due to reduction in NO_3^- content and NR activity, thus internal nitrite (NO_2^-) concentration decreased as NiR is responsible for NO_2^- reduction to NH_4^+. However, application of exogenous NaHS can enhance the NiR activity as well the transcript abundance of this enzyme encoding gene Os*NiR*, which is validated by enhancement in NO_3^- concentration in rice leaves when exposed to Ni stress conditions.

In the present study, we reported the enhancement in NH_4^+ concentration in rice leaves when exposed to Ni stress. The increase in NH_4^+ concentration is parallel with the upregulation of the transcript levels of Os*AMT1;1* (Fig. 6b). Gajewska et al. (2009) reported that NH_4^+ concentration was increased in wheat shoots in response to high Ni doses. Increase accumulation of NH_4^+ within cells is toxic and caused various damages, such as intracellular pH alteration, disturbance in osmotic balance, ATP synthesis inhibition, nutrient deficiency and necrosis (Gerendás et al., 1997). Thus, under Ni-challenged plants, enhancement in NH_4^+ concentration may be related to reduced growth of rice seedlings (Table 1 and Fig. 3c). However, addition of NaHS together with Ni remarkably decreased the NH_4^+ contents in rice leaves, as compared to Ni-treated only seedling. These results indicate an accurate fusion of NH_4^+ into glutamate, which is validated by enhanced growth. NH_4^+ assimilating enzymes such as GOGAT, GDH and GS play vital roles in plant growth and development. GS transforms NH_4^+ into glutamine, which is then converted into glutamate by GOGAT (Ireland and Lea, 1999). Furthermore, NH_4^+ can directly be converted to glutamate by GDH under environmental stress conditions, when GS/GOGAT cycle pathway is inhibited (Yang et al., 2013). Our results prove that GS and GOGAT activities were inhibited (Fig. 5e and f), and the expression levels of their associated genes (*OsGS2, OsFd-GOGAT* and *OsNADH-GOGAT*) were downregulated in response to Ni stress compared to control. These findings proposed that inhibition in the activities of GS and GOGAT disturbed NH_4^+ assimilation process, which is validated by decreased NO_3^- content and increased NH_4^+ content. Furthermore, reduction in GS and GOGAT activity has been ascribed to oxidative variations of these enzymes under stress conditions. Our results are in accordance with other workers, who reported an inhibition in GS activity in rice shoot (Mishra and Dubey, 2011), and a decreased in Fd-GOGAT activity in wheat shoot (Gajewska et al., 2009). GDH activity was enhanced in response to Ni toxicity (Fig. 5g), and the abundance of *OsGDH1* expression was upregulated (Fig. 6h), and these findings coincide with the results of Gajewska et al. (2009) in wheat shoots under Ni stress. However, NaHS in Ni combination significantly enhanced the activities of GS and GOGAT and expression levels of *OsGS2, OsFd-GOGAT* and *OsNADH-GOGAT*, while suppressing GDH activity and expression level of *OsGDH1*. These results clearly demonstrate the alleviating role of NaHS against Ni toxicity in rice seedling by exerting a favorable impact on N metabolism. Moreover, transamination reactions are vital for plant N metabolism, because it transfers amino groups from glutamate to other amino acids. Liang et al. (2011) explained that GOT and GPT are the supreme active aminotransferases, which catalyze the various reactions that produce aspartate and alanine, respectively. In this study, we found that GOT and GPT activities were inhibited under Ni stress in rice leaves (Fig. 5h and i). It was suggested that decline in GOT and GPT activities under stress conditions may be linked by the weakened GS/GOGAT pathway, as glutamate is the first amino acid synthesized from inorganic N taken up by plants (Liang et al., 2011), and is primarily derived from NH_4^+ assimilation catalyzed by GS and GOGAT (Gangwar and Singh, 2011). However, addition of NaHS together with Ni significantly elevated the activities of GOT and GPT in rice leaves compared to Ni-challenged seedlings alone (Fig. 5h and i). These results suggest that exogenous application of NaHS improved the GS/GOGAT pathway and produced more glutamate, which is a substrate of transamination reactions.

5. Conclusion

To sum up, this is the first report demonstrating the relationship between H_2S and the system of N metabolism for alleviating the effects of Ni stress in plants. Our results suggested that Ni stress induces detrimental effects on photosynthesis, chloroplast ultrastructure, and N metabolism, but combined application of NaHS facilitate the seedlings to combat the harmful effects of Ni through the improvement of photosynthesis and maintain the nitrogen metabolism both at activity as well as genetic level, which eventually contribute to the improvement of rice growth performance under Ni stress.

Conflicts of interest

The authors declare no conflicts of interest.

Author's contribution

M.R. and S.T. conceived, designed the experiments. M.R. and M.G.M. wrote the manuscript. M.R. M.Z.A, S.M., O.M. and M.I[1]. contributed to collect data and analysis the experiments. Y.Z., M.A., M.A.A., and R.J. contributed with the data analysis and with the critical revision of the manuscript. M.I[2] and YL helped in the revision of manuscript. S.T. has supervised the experiments as well as guidance through all work stages.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.02.023.

References

Ali, S., Farooq, M.A., Hussain, S., Yasmeen, T., Abbasi, G., Zhang, G., 2013. Alleviation of chromium toxicity by hydrogen sulfide in barley. Environ. Toxicol. Chem. 32, 2234–2239.
Boussama, N., Ouariti, O., Ghorbal, M.H., 1999. Changes in growth and nitrogen assimilation in barley seedlings under cadmium stress. J. Plant Nutr. 22, 731–752.
Cameron, K.C., Haynes, R.J., 1986. Retention and movement of nitrogen in soils. In: Haynes, R.J. (Ed.), Mineral Nitrogen in the Plant-Soil System. Academic Press, New York, pp. 166–241.
Chen, C., Huang, D., Liu, J., 2009. Functions and toxicity of nickel in plants: recent advances and future prospects. Clean. - Soil, Air, Water 37, 304–313.
Chen, J., Wang, W.H., Wu, F.H., You, C.Y., Liu, T.W., Dong, X.J., He, J.X., Zheng, H.L., 2013. Hydrogen sulfide alleviates aluminum toxicity in barley seedlings. Plant Soil 362, 301–318.
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.
Christou, A., Filippou, P., Manganaris, G.A., Fotopoulos, V., 2014. Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. BMC Plant Biol. 14, 42.
Christou, A., Manganaris, G.A., Papadopoulos, I., Fotopoulos, V., 2013. Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. J. Exp. Bot. 64, 1953–1966.
Dawood, M., Cao, F., Jahangir, M.M., Zhang, G., Wu, F., 2012. Alleviation of aluminum toxicity by hydrogen sulfide is related to elevated ATPase, and suppressed aluminum uptake and oxidative stress in barley. J. Hazard Mater. 209, 121–128.
de Macedo, F.G., Bresolin, J.D., Santos, E.F., Furlan, F., da Silva, W.T.L., Polacco, J.C., Lavres, J., 2016. Nickel availability in soil as Influenced by liming and its role in soybean nitrogen metabolism. Front. Plant Sci. 7, 1358.
Dooley, F.D., Nair, S.P., Ward, P.D., 2013. Increased growth and germination success in plants following hydrogen sulfide administration. PLoS One 8 (4) e62048.
Duan, B., Ma, Y., Jiang, M., Yang, F., Ni, L., Lu, W., 2015. Improvement of photosynthesis in rice (*Oryza sativa* L.) as a result of an increase in stomatal aperture and density by exogenous hydrogen sulfide treatment. Plant Growth Regul. 75, 33–44.
Erdal, S., Turk, H., 2016. Cysteine-induced upregulation of nitrogen metabolism-related genes and enzyme activities enhance tolerance of maize seedlings to cadmium stress. Environ. Exp. Bot. 132, 92–99.
Fabiano, C.C., Tezotto, T., Favarin, J.L., Polacco, J.C., Mazzafera, P., 2015. Essentiality of nickel in plants: a role in plant stresses. Front. Plant Sci. 6, 754.
Gajewska, E., Wielanek, M., Bergier, K., Skłodowska, M., 2009. Nickel-induced depression of nitrogen assimilation in wheat roots. Acta Physiol. Plant. 31, 1291.
Gangwar, S., Singh, V.P., 2011. Indole acetic acid differently changes growth and nitrogen metabolism in *Pisum sativum* L. seedlings under chromium (VI) phytotoxicity: implication of oxidative stress. Sci. Hortic. 129, 321–328.
García-Mata, C., Lamattina, L., 2010. Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. New Phytol. 188, 977–984.
Gerendás, J., Zhu, Z., Bendixen, R., Ratcliffe, R.G., Sattelmacher, B., 1997. Physiological and biochemical processes related to ammonium toxicity in higher plants †. Z. für Pflanzenernährung Bodenkunde 160, 239–251.
Gratao, P.L., Pompeu, G.B., Capaldi, F.R., Vitorello, V.A., Lea, P.J., Azevedo, R.A., 2008. Antioxidant response of *Nicotiana tabacum* cv. Bright Yellow 2 cells to cadmium and nickel stress. Plant Cell Tiss. Org. 94, 73–83.
Hassan, M.J., Zhang, G., Zhu, Z., 2008. Influence of cadmium toxicity on plant growth and nitrogen uptake in rice as affected by nitrogen form. J. Plant Nutr. 31, 251–262.
Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J. Exp. Bot. 58, 2369–2387.
Hou, Z., Wang, L., Liu, J., Hou, L., Liu, X., 2013. Hydrogen sulfide regulates ethylene-induced stomatal closure in *Arabidopsis thaliana*. J. Integr. Plant Biol. 55, 277–289.
Ireland, R.J., Lea, P.J., 1999. The Enzymes of Glutamine, Glutamate, Asparagine and Aspartate Metabolism. Contribution in Book/report/proceedings.
Jin, Z., Shen, J., Qiao, Z., Yang, G., Wang, R., Pei, Y., 2011. Hydrogen sulfide improves drought resistance in *Arabidopsis thaliana*. Biochem. Biophys. Res. Commun. 414, 481–486.
Khaliq, A., Ali, S., Hameed, A., Farooq, M.A., Farid, M., Shakoor, M.B., Mahmood, K., Ishaque, W., Rizwan, M., 2016. Silicon alleviates nickel toxicity in cotton seedlings through enhancing growth, photosynthesis, and suppressing Ni uptake and oxidative stress. Arch. Agron Soil Sci. 22, 633–647.
Kutman, B.Y., Kutman, U.B., Cakmak, I., 2014. Effects of seed nickel reserves or externally supplied nickel on the growth, nitrogen metabolites and nitrogen use efficiency of urea-or nitrate-fed soybean. Plant Soil 376, 261–276.
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.
Lawlor, D.W., 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. J. Exp. Bot. 53, 773–787.
Li, J., Jia, H., Wang, J., Cao, Q., Wen, Z., 2014a. Hydrogen sulfide is involved in maintaining ion homeostasis via regulating plasma membrane Na+/H+ antiporter system in the hydrogen peroxide-dependent manner in salt-stress *Arabidopsis thaliana* root. Protoplasma 251, 899–912.
Li, L., Wang, Y., Shen, W., 2012. Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots. Biometals 25, 617–631.
Li, Z.G., Yi, X.Y., Li, Y.T., 2014b. Effect of pretreatment with hydrogen sulfide donor sodium hydrosulfide on heat tolerance in relation to antioxidant system in maize (*Zea mays*) seedlings. Biologia 69, 1001–1009.
Liang, C.G., Chen, L.P., Wang, Y., Liu, J., Guang-Li, X.U., Tian, L.I., 2011. High temperature at grain-filling stage affects nitrogen metabolism enzyme activities in grains and grain nutritional quality in rice. 水稻科学(英文版) 18, 210–216.
Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of Total Carotenoids and Chlorophylls a and B of Leaf Extracts in Different Solvents. Portland Press Limited.
Lisjak, M., Teklic, T., Wilson, I.D., Whiteman, M., Hancock, J.T., 2013. Hydrogen sulfide: environmental factor or signalling molecule? Plant Cell Environ. 36, 1607–1616.
Lisjak, M., Teklić, T., Wilson, I.D., Wood, M., Whiteman, M., Hancock, J.T., 2011. Hydrogen sulfide effects on stomatal apertures. Plant Signal. Behav. 6, 1444–1446.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25, 402–408.
Luo, J., Li, H., Liu, T., Polle, A., Peng, C., Luo, Z.B., 2013. Nitrogen metabolism of two contrasting poplar species during acclimation to limiting nitrogen availability. J. Exp. Bot. 64, 4207–4224.
Meng, J.F., Xu, T.F., Wang, Z.Z., Fang, Y.L., Xi, Z.M., Zhang, Z.W., 2014. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. J. Pineal Res. 57, 200–212.
Mishra, P., Dubey, R., 2011. Nickel and Al-excess inhibit nitrate reductase but upregulate activities of aminating glutamate dehydrogenase and aminotransferases in growing rice seedlings. Plant Growth Regul. 64, 251–261.
Mosa, A., El-Banna, M.F., Gao, B., 2016. Biochar filters reduced the toxic effects of nickel on tomato (*Lycopersicon esculentum* L.) grown in nutrient film technique hydroponic system. Chemosphere 149, 254–262.
Mostofa, M.G., Fujita, M., Tran, L.-S.P., 2015a. Nitric oxide mediates hydrogen peroxide- and salicylic acid-induced salt tolerance in rice (*Oryza sativa* L.) seedlings. Plant Growth Regul. 77, 265–277.
Mostofa, M.G., Rahman, A., Ansary, M.M.U., Watanabe, A., Fujita, M., Tran, L.-S.P., 2015b. Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. Sci. Rep. 5, 14078.
Polacco, J.C., Mazzafera, P., Tezotto, T., 2013. Opinion–Nickel and urease in plants: still many knowledge gaps. Plant Sci. 199, 79–90.
Rehman, Z.U., Rizwan, M., Ali, S., Fatima, N., Yousaf, B., Naeem, A., Sabir, M., Ahmad, H.R., Yong, S.O., 2016. Contrasting effects of biochar, compost and farm manure on alleviation of nickel toxicity in maize (*Zea mays* L.) in relation to plant growth, photosynthesis and metal uptake. Ecotoxicol. Environ. Saf. 133, 218–225.
Rizwan, M., Imtiaz, M., Dai, Z., Mehmood, S., Adeel, M., Liu, J., Tu, S., 2017. Nickel stressed responses of rice in Ni subcellular distribution, antioxidant production, and osmolyte accumulation. Environ. Sci. Pollut. Res. 24, 20587–20598.
Saad, R., Kobaissi, A., Robin, C., Echevarria, G., Benizri, E., 2016. Nitrogen fixation and growth of *Lens culinaris* as affected by nickel availability: a pre-requisite for optimization of agromining. Environ. Exp. Bot. 131, 1–9.
Savvides, A., Ali, S., Tester, M., Fotopoulos, V., 2016. Chemical priming of plants against multiple abiotic stresses: mission possible? Trends Plant Sci. 21, 329–340.
Sharma, P., Dubey, R.S., 2005. Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. J. Plant Physiol. 162, 854–864.
Shukla, R., Gopal, R., 2009. Excess nickel alters growth, metabolism, and translocation of

certain nutrients in potato. J. Plant Nutr. 32, 1005–1014.
Siddiqui, M.H., Al-Whaibi, M.H., Basalah, M.O., 2011. Interactive effect of calcium and gibberellin on nickel tolerance in relation to antioxidant systems in *Triticum aestivum* L. Protoplasma 248, 503–511.
Singh, V.P., Singh, S., Kumar, J., Prasad, S.M., 2015. Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate–glutathione cycle: possible involvement of nitric oxide. J. Plant Physiol. 181, 20–29.
Sirhindi, G., Mir, M.A., Abd-Allah, E.F., Ahmad, P., Gucel, S., 2016. Jasmonic Acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in *Glycine max* under nickel toxicity. Front. Plant Sci. 7, 591.
Soares, C., de Sousa, A., Pinto, A., Azenha, M., Teixeira, J., Azevedo, R.A., Fidalgo, F., 2016. Effect of 24-epibrassinolide on ROS content, antioxidant system, lipid peroxidation and Ni uptake in *Solanum nigrum* L. under Ni stress. Environ. Exp. Bot. 122, 115–125.
Wang, Y., Li, L., Cui, W., Xu, S., Shen, W., Wang, R., 2012. Hydrogen sulfide enhances alfalfa (*Medicago sativa*) tolerance against salinity during seed germination by nitric oxide pathway. Plant Soil 351, 107–119.
Yang, Y., Lu, X., Yan, B., Li, B., Sun, J., Guo, S., Tezuka, T., 2013. Bottle gourd rootstock-grafting affects nitrogen metabolism in NaCl-stressed watermelon leaves and enhances short-term salt tolerance. J. Plant Physiol. 170, 653–661.
Yoshida, S., Forn, D.A., Sock, J.H., Gomez, K.A., 1976. Laboratory Manual for Physiological Studies of Rice, third ed. International Rice Research Institute, Manila, Philippines.
Yuan, L., Shu, S., Sun, J., Guo, S., Tezuka, T., 2012. Effects of 24-epibrassinolide on the photosynthetic characteristics, antioxidant system, and chloroplast ultrastructure in *Cucumis sativus* L. under $Ca(NO_3)_2$ stress. Photosynth. Res. 112, 205–214.
Zhang, H., Tan, Z.Q., Hu, L.Y., Wang, S.H., Luo, J.P., Jones, R.L., 2010. Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. J. Integr. Plant Biol. 52, 556–567.
Zhang, H., Tang, J., Liu, X.P., Wang, Y., Yu, W., Peng, W.Y., Fang, F., Ma, D.F., Wei, Z.J., Hu, L.Y., 2009. Hydrogen sulfide promotes root organogenesis in *Ipomoea batatas, Salix matsudana* and *Glycine max*. J. Integr. Plant Biol. 51, 1086–1094.
Zhang, Y., Liu, Z., Liu, R., Wang, L., Bi, Y., 2011. Gibberellins negatively regulate light-induced nitrate reductase activity in Arabidopsis seedlings. J. Plant Physiol. 168, 2161–2168.