Hydrogen Sulphide Improves Iron Homeostasis in Wheat Under Iron-Deficiency

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Abstract: Hydrogen sulphide (H\(_2\)S) is emerging as an important gaseous molecule involved in various plant developmental processes and plant stress responses. In this study, exogenous H\(_2\)S donor (sodium hydrosulfide, NaHS) treated wheat plants were used to investigate the role of H\(_2\)S in response to iron-deficiency. The results showed that H\(_2\)S significantly alleviated leaf chlorosis under iron-deficient conditions, and thus improved photosynthesis. Moreover, H\(_2\)S increased the lateral root (LR) number, density and length of wheat seedlings grown in iron-sufficient and deficient culture solution, and promoted phytosiderophores (PSs) secretion from roots simultaneously, which eventually led to an increase in iron uptake. Taken together, these results indicate that H\(_2\)S improved iron uptake by regulating root development and PSs secretion, and consequently increased chlorophyll biosynthesis and photosynthesis in plants under iron-deficiency.

Keywords: Hydrogen Sulfide, Iron-Deficiency, Phytosiderophores, Lateral Root, Wheat

1. Introduction

Iron (Fe) is an essential nutrient for all organisms, due to its important role in various cellular metabolic processes. Although the abundance of iron in soils is high, iron availability in a form that can be absorbed and used by plants is limited. It has been estimated that up to 40% of the world's arable land may be iron-deficient [1], and the problem is particularly serious in alkaline soils, as iron availability decreases with increasing soil pH [2]. Iron-deficiency in plants has become a worldwide concern. It usually causes a decrease in chloroplast particles and thylakoid lamellae, disorders in the basal thymid and matrix thylakoid arrangement, and even leads to chloroplast dissociation. Iron is an important participant in the redox reaction and electron transport in plants, therefore, iron-deficiency usually weakens photosynthesis and respiration, thus affecting plant yield and quality. Iron has been shown to be a limiting factor for biomass in important crop plants, such as tomato [3], spinach [4], and rice [5].

In order to adapt to iron-deficiency conditions, plants usually regulate the processes of iron absorption and transport. Specifically, under iron-deficiency, Strategy I plants secrete protons to the rhizosphere by a plasma membrane H\(^+\)-ATPase, thereby reducing the soil pH and improving iron availability. Subsequently, the ferric-chelate reductase (FCR) on the plasma membrane of root epidermal cells reduces Fe (III) to Fe (II), and then Fe (II) is transported to the cells through a specific Fe transporter. Strategy II plants (graminaceous species) increase iron absorption by inducing the release of phytosiderophores (PSs) in roots, which can effectively chelate Fe (III) around the rhizosphere to form Fe (III)-PS complex. This Fe (III)-PS complex is then transported into the cell through special membrane transporters (like YS and YSL) on the root surface [6]. Additionally, the processes of iron absorption and transport are regulated by plant hormones and signal molecules. Exogenous nitric oxide (NO) enhances iron availability and alleviates iron-deficiency symptoms in maize under low iron conditions [7]; Carbon monoxide (CO) facilitates the absorption of iron in Arabidopsis thaliana [8]; Auxin promotes the activity of ferric-chelate reductase in soybean [9], cucumber [10] and clover [11]; Ethylene
regulates the expression of iron transporter (IRT1) and ferric-chelate reductase (FRO2) by reducing FIT1 protein degradation [12].

Apart from NO and CO, H$_2$S is also an important gas signaling molecule [13-15], and its physiological function in plants has been extensively studied in recent years. H$_2$S regulates the physiological processes of plant stomatal closure, seed germination, root development and senescence [16-19], and it also induces responses to various abiotic stresses (e.g. drought, heavy metals, high temperature, etc.) [20-24]. Moreover, H$_2$S is also involved in NO, auxin and ethylene signaling pathways. For instance, NO enhances the resistance of Cynodon dactylon to cadmium (Cd) stress by inducing H$_2$S production [25]; Auxin regulates lateral root development in tomato and Arabidopsis thaliana by inducing H$_2$S [26-27]. Finally, H$_2$S also mediates the ethylene-regulated stomatal closure in Arabidopsis thaliana [28].

Iron-deficiency leads to plant chlorosis, chlorophyll reduction, and photosynthesis inhibition. Studies have shown that H$_2$S can enhance rice and spinach photosynthesis under normal conditions [29-30], as well as improve chlorophyll fluorescence of strawberry and wheat when exposed to drought stress [24, 31]. Despite the documented involvement of H$_2$S in NO, auxin and ethylene signaling pathways, all of which induce responses to iron-deficiency, it is unclear whether H$_2$S participates in the regulation of plant iron nutrition. However, H$_2$S has been showed to promote iron uptake in rice roots and leaves under both normal conditions and Cd stress [32]. On the basis of these results, the effects of H$_2$S on wheat growth, chlorophyll content, photosynthesis, PSs release, and Fe content in wheat under Fe-deficient and sufficient conditions were investigated. Wheat was considered to be a research object because it is an important crop and a Strategy II plant, which releases PSs under Fe shortage.

2. Methods and Materials

2.1. Plant Growth and Treatments

The seeds of common cultivated wheat varieties Aikang 58 were surface-sterilized and germinated on water soaked filter papers for 48 h, and then grown in 1/2 strength Hoagland nutrient solution. Three days later, the seedlings were transferred to Fe-sufficient (50 mM) and deficient (0 or 1 mM) nutrient solution with or without 0.4 mM NaHS, and the nutrient solution was changed every two days. Therefore, depending on treatment, four groups of wheat seedlings were included in this study: Fe-sufficiency and no NaHS treatment as the control group (+Fe), Fe-sufficiency and NaHS treatment (+Fe+NaHS), Fe-deficiency and no NaHS treatment (-Fe), Fe-deficiency and NaHS treatment (-Fe+NaHS). After two weeks, the third leaves were used for detecting chlorophyll content and photosynthesis (Pn), and all the leaves were used for H$_2$S and Fe content assays. The seedlings grew under the conditions of 25/18°C (light/dark), 300 µmol m$^{-2}$ s$^{-1}$ light intensity, 70% humidity and a 14 h photoperiod.

2.2. Determination of Chlorophyll Content and Photosynthesis (Pn)

Total chlorophyll was extracted from leaves with 80% acetone until complete bleaching, and the content of chlorophyll was then calculated from the absorbance at 470, 646, and 663 nm, following Lichtenthaler [33]. Pn was measured using a portable photosynthesis system (LI-6400, NE, USA) on the third fully developed leaf of the seedlings.

2.3. Collection of Root Exudates and Determination of PSs Release

PSs release from seedling roots was analysed by determining PSs content in root washing. After treatment, the roots of wheat seedlings were washed twice with deionized water, and then root systems were transferred to 200 ml deionized water for 6 h. This solution of 200 ml was used as a sample to determine PSs content released from roots according to the Fe-binding assay as described by Reichman and Parker [34].

2.4. Detection of H$_2$S Content

H$_2$S quantification was performed as described by Christou [31]. Wheat leaves were powdered with liquid nitrogen, and H$_2$S was extracted using 100 mM potassium phosphate buffer (pH 7) containing 10 mM ethylene diamine tetraacetic acid (EDTA). After centrifugation of 12,000 g at 4°C for 15 min, 100 µl of the supernatant was added to an assay mixture containing 1.88 ml of extraction buffer and 20 µl of 20 mM 5, 5′-dithiobis (2-nitrobenzoic acid). The absorbance was measured at 412 nm after a 2 min incubation time at room temperature. NaHS was used for standard curve.

2.5. Iron Quantification

Harvested seedlings were washed twice in 5 mM CaSO$_4$ and 10 mM EDTA solution and then dried at 70°C. The samples with a weight of at least 0.2 g were digested completely in 70% HNO$_3$ at 120°C. Finally, the solution was diluted to a certain volume with distilled deionized water. Iron was quantified with inductively coupled plasma spectrometry (Perkin Elmer Optimal 2100DV).

2.6. Statistical Analysis

All data were collected from at least three independent experiments. For photosynthesis measurement, at least six leaves were used. For other analyses, the results were the mean of three replicated treatments. Differences of the means among treatments were compared using Duncan’s multiple range tests at 0.05 probability level.
3. Results

3.1. Phenotype, Chlorophyll Content and Photosynthesis in Wheat Leaves

Iron-deficiency affected chlorophyll synthesis, and resulted in leaf chlorosis. However, exogenous sodium hydrosulfide (NaHS, an H\(_2\)S donor) treatment significantly alleviated plant chlorosis and increased chlorophyll content under iron-deficiency (Figure 1). Naturally, the reduction of chlorophyll caused a decline in photosynthesis under Fe-deficiency. Wheat seedlings under Fe-deficiency showed a 67.2% reduction in photosynthesis compared to the control group. However, that reduction was limited under treatment with H\(_2\)S, dropping to 51.4% of that in the control.

![Figure 1. Effect of NaHS on phenotype (a), chlorophyll content (b) and photosynthesis (c) of iron-deficient wheat leaves. Wheat seedlings were grown in Fe-sufficient (50 mM) and deficient (0 mM) nutrient solution with or without 0.4 mM NaHS treatment for two weeks. After that, the third leaves were used to assay. Values are the means ± standard deviation (SD). Different letters denote significant differences between treatments (P < 0.05).](image)

3.2. Lateral Root (LR) Formation

Fe-deficiency usually causes changes in plant lateral roots. Therefore, several parameters of wheat lateral root (LR) formation, namely lateral root number, density and length were investigated. As shown in Figure 2, under iron-deficiency for two weeks, the number, density and length of LR increased significantly. Exogenously applied NaHS further exacerbated this increase under both Fe-deficient and sufficient conditions. In comparison with the no-NaHS treatment group, treatment with NaHS increased the number, density and length of lateral roots. The increase was of 40.4%, 61.4%, 57.2%, respectively, under Fe-sufficiency, and of 32.7%, 263.0%, 60.3%, respectively, in Fe-deficient conditions.
3.3. Fe Content and Phytosiderophores (PSs) Release

Fe content significantly decreased in leaves and roots under Fe-limited conditions, while NaHS treatment improved iron accumulation under both Fe-sufficient and limited conditions (Table 1). In comparison with the no-NaHS treatment group, NaHS application promoted iron accumulation in leaves (1.73 times) and roots (1.43 times) under Fe-sufficiency. Iron accumulation increased also under Fe-limited conditions both in leaves (1.51 times) and roots (1.90 times).

The secretion of PSs from roots promotes iron absorption and, therefore, the content of PSs released by roots increased significantly under conditions of iron-deficiency, as expected. The increase was up to 1.75 times compared to the control group, and up to 2.28 times under exposure to NaHS. Even under Fe-sufficient conditions, NaHS also increased PSs secretion by 1.42 times compared to the control group (Table 1).

Table 1. Effect of NaHS on Fe content and PSs release in iron-deficient wheat plants. Values are the means ± standard deviation (SD). Different letters denote significant differences between treatments ($P < 0.05$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fe content (mg g⁻¹ DW)</th>
<th>PSs release (nmol g⁻¹ RFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Root</td>
</tr>
<tr>
<td>+Fe</td>
<td>0.081±0.006b</td>
<td>3.131±0.394b</td>
</tr>
<tr>
<td>+Fe+NaHS</td>
<td>0.140±0.029a</td>
<td>4.486±0.412a</td>
</tr>
<tr>
<td>-Fe</td>
<td>0.069±0.007c</td>
<td>0.216±0.038d</td>
</tr>
<tr>
<td>-Fe+NaHS</td>
<td>0.104±0.019ab</td>
<td>0.411±0.079c</td>
</tr>
</tbody>
</table>
3.4. H$_2$S Content in Shoots and Roots

H$_2$S concentration was determined in both shoots and roots of wheat plants grown in Fe-sufficient and limited culture solutions (Figure 3). The H$_2$S content increased in leaves (145%) and decreased in roots (50%) under Fe-limited conditions compared to the Fe-sufficient group. In comparison with the no-NaHS treatment group, exogenously applied NaHS increased H$_2$S content in leaves and roots under both Fe-limited and sufficient conditions. The increase was of 21.2% in leaves and 253.6% in roots under Fe-sufficiency, and of 41.9% and 307.1% respectively, in Fe-limited conditions.

![Figure 3. H$_2$S content in iron-deficient wheat leaf (a) and root (b). Wheat seedlings were grown in Fe-sufficient (50 mM) and deficient (1 mM) nutrient solution with or without 0.4 mM NaHS treatment for two weeks. After that, all the leaves and roots were used to assay respectively. Values are the means ± standard deviation (SD). Different letters denote significant differences between treatments (P < 0.05).](image)

4. Discussion

Iron is one of the most important elements for plant growth, especially for chloroplast development, photosynthesis, respiration and DNA synthesis. Leaf yellowing is a typical phenotype caused by Fe-deficiency and first exhibits itself in young leaves. This was also shown in this study, where the content of chlorophyll in the youngest leaf (the third leaf) of wheat seedlings significantly decreased under Fe-deficiency conditions (Figure 1a, b). As chlorophyll is the basis of photosynthesis in plants, it is not surprising that, with chlorophyll content decreased, Fe-deficiency naturally caused a significant decline in photosynthesis (Figure 1c). However, exogenous NaHS treatment significantly increased chlorophyll synthesis and photosynthetic rate in wheat leaves under Fe-deficiency, thus alleviating the effects of Fe-deficiency (Figure 1). These findings that H$_2$S can regulate chlorophyll synthesis and photosynthetic rate are supported by previous studies. For example, H$_2$S enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings [29]; H$_2$S can regulate plant growth and photosynthesis under Cr and Pd stress in barley and cotton, respectively [35-36]; A treatment with low concentration of NaHS can improve photosynthesis in rice by increasing its stomatal aperture and density [30].

Plants induce a variety of morphological and physiological changes to adapt to Fe-limited conditions [37]. One of those changes is the increase of lateral root (LR) development, which has been demonstrated in Arabidopsis and tomato [38-39]. Jin et al. [40] showed that the increase of root branching contributes to enhanced activity of the root ferric chelate reductase under iron-deficiency. Another study showed that NO acts downstream of auxin in regulating Fe-deficiency-induced root branching that enhances Fe-deficiency tolerance in tomato [41]. Recently, the role of H$_2$S in lateral root development has also been investigated. NaHS treatment induced pepper lateral root formation, while hypotaurine (H$_2$S scavenger) showed an adverse effect [42]. H$_2$S inhibits primary root, LR and root hair elongation but promotes LR initiation in Arabidopsis thaliana seedlings [27]. Further, auxin depletion-induced inhibition of lateral root formation is rescued by NaHS [26]. In this study, NaHS treatment significantly increased lateral root number, density and length under both Fe-deficiency and sufficiency conditions. These results, combined with previous findings, lead to the conclusion that the function of H$_2$S on improving wheat Fe-deficiency was related to the regulation of lateral root development.

Phytosiderophores (PSs) secretion from roots is known to occur under Fe-deficiency for Strategy II plants. Studies on grain crops and other grasses have shown that Fe-deficiency tolerance was strongly correlated to PSs content, which may facilitate iron absorption by forming ferric-phytosiderophore [43-46]. The results in this study lend support to this idea, as the increased PSs secretion induced by H$_2$S corresponded to higher iron accumulation in wheat leaves and roots under both Fe-deficient and sufficient conditions (Table 1).

H$_2$S, as a signal molecule, is known to regulate plant growth and development, and also induce responses to various stresses. In this study, results showed that H$_2$S content differed under Fe-limited and sufficient conditions (Figure 3). Low iron induced H$_2$S production in wheat leaves but decreased it in roots, while PSs secretion from roots increased under Fe-limited conditions. Moreover, the increase of H$_2$S content caused by exogenous NaHS in leaves was also accompanied by the increase of PSs secretion in wheat roots. Previous studies have proposed that plants’ Fe-deficiency response was
related to sensors which are localized in leaves and roots [2, 47]. The results of this study, combined, suggest that H₂S concentration in shoots might play a major role in response to Fe-deficiency, potentially by being a sensor in the leaves that responds to Fe-deficiency.

5. Conclusion

The findings of this study showed that H₂S plays an important role in the response of wheat plants to Fe-deficiency. In particular, H₂S can improve PSs secretion and iron uptake, consequently increasing chlorophyll biosynthesis and photosynthesis. Although this study provides important insights into the role of H₂S in plants’ response to Fe-deficiency, further studies are needed to understand the regulatory mechanism of H₂S on PSs secretion and iron uptake.

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References


