

Title: Hydrogen sulfide improves adaptation of *Zea mays* seedlings to iron deficiency

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Table S1 Sequences of forward and reverse primers used in qRT-PCR for gene expression analysis in maize seedlings.

Gene name	Full name	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
<i>ZmMTN</i>	S-adenosylhomocysteine nucleosidase	ACATAACAAGCATCGGATCG	GGATGAGGATTAGGTCTACA
<i>ZmNAS1</i>	nicotianamine synthase 1	CGCTGCTTACAGGAGTCCTA	TATTATCAATGTTTAGCGAC
<i>ZmNAS3</i>	nicotianamine synthase 3	TGCGTACACCACATCTGTGC	AATCGTACATCGAATCGAG
<i>ZmDMAS1</i>	deoxymugineic acid synthase 1	AGCTTCGAGAAGTGCTAGTC	TTACGCACGGTGACGGGACG
<i>ZmTOM1</i>	transporter of MAs 1	GTGCCGTACTTAAATGTGAA	ACGGAAGAAAGGTCCCCTAG
<i>ZmTOM2</i>	transporter of MAs 2	TACCTATCGCGGCTGCTTTG	GCGGTCGTACGGCGCAAGAA
<i>ZmTOM3</i>	transporter of MAs 3	TGCGGACACTGACTTTCGTG	TTGTTTTAGGAACCTGGATA
<i>ZmNRAMP1</i>	natural resistance associated macrophage protein	TTGCTGGGGTACTAAACGAG	CCTTGTCACGACATCTAGGT
<i>ZmMATE2</i>	multi-drug and toxin efflux 2	AGTTTCATCTTCGGATCCTC	CCAATGTAAGTCTGGTTGA
<i>ZmFRO1</i>	ferric-chelate reductase 1	TTCGCTCTTCCAACCTCCAACCTTCTGT	TTGATTACTTCTTCACCTGTAGCGTCT
<i>ZmIBP</i>	iron binding protein	AACGGCTGCCTCAGTGAAGAGAT	CAAGCGATATGACGAGGTGATTCTGT
<i>ZmIRT</i>	iron-regulated transporter 1	CCACCGTGTTCATCGCTCAGGTTCTTG	TAGACCCATGCCCTCGAACATTTGG
<i>ZmYS1</i>	yellow stripe 1	CACGGAAATGGAGAATGCGAGATAC	ACATAGGTTGAACCAACATGACAA
<i>ZmST1</i>	sulfate transporter 1	TTGGCAGACAATACTGATAGGAGCG	ATTAGACTAGCTGAAGGTGGGTTGA
<i>ZmATPS</i>	ATP sulfurylase	CCCAGAATCAACTGTTGTTGCGATC	TCCCGTGGTCAGCATCATAGAGGT

<i>ZmAPR</i>	adenosine 5'-phosphosulfate reductase	GGCTCCGAAATCGCCATCGCCTTCAG	TGCCGTAGTGCTTCTCCACCTTGTCG
<i>ZmOASTL1</i>	<i>O</i> -acetyl-L-serine(thiol)lyase 1	TCACCCAGCTCATCGGCAACAC	ACGCTACAGCAGGGCTCCATAATCT
<i>ZmOASTL2</i>	<i>O</i> -acetyl-L-serine(thiol)lyase 2	TCCAACCTCCGACGAGAACCGCCAC	CGACGCACCCATCGGTACACCTTGTT
<i>ZmDES</i>	Cys desulfhydrase	TGAGATCCTGAGCTATGCTATGGTC	GGAGGCTGCTTCTTTAGGCTTTGA
<i>ZmRBCL</i>	ribulose-1,5-bisphosphate carboxylase large subunit	ACCTATTTGAAGAGGGTTCTGTTAC	GATACCGTGAGGCGGACCTTGGA
<i>ZmRBCS</i>	ribulose-1,5-bisphosphate carboxylase small subunit	GCAGTGCCTCAGCTTCATCGCCTACAA	TCGACAACATCAAGCAGACGCAGTG
<i>Zmpsba</i>	D1 protein	TTTACATTGGATGGTTCGGTGTTTTGA	ATCTACTGGAGGAGCAGCGATGAAGG
<i>ZmPEPC</i>	phosphoenolpyruvate carboxylase	CCATCTGCTGGCTTCTGGAGTTTC	ACCAGGGAGAGCCCGAACGTGAAAA
<i>Zmactin2</i>	actin 2	TTGATTATGAACAGGAGCTGGAGACT	AGCCACCACTGAGGACAACATTACC

Table S2 Procedures of dsDNA synthesis used in qRT-PCR for gene expression analysis in leaves of maize plant.

Gene name	Procedures of dsDNA synthesis
<i>ZmMTN</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 46 °C for 30 s and 72 °C for 20 s
<i>ZmNAS1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 49 °C for 30 s and 72 °C for 20 s
<i>ZmNAS3</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 51 °C for 30 s and 72 °C for 20 s
<i>ZmDMAS1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 20 s
<i>ZmTOM1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 61 °C for 30 s and 72 °C for 20 s
<i>ZmTOM2</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 53 °C for 30 s and 72 °C for 25 s
<i>ZmTOM3</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 24 s
<i>ZmNRAMP1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 51 °C for 30 s and 72 °C for 20 s
<i>ZmMATE2</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 20 s
<i>ZmFRO1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 20 s
<i>ZmIBP</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 57 °C for 30 s and 72 °C for 20 s
<i>ZmIRT</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 20 s
<i>ZmYS1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 53 °C for 30 s and 72 °C for 20 s
<i>ZmST1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 20 s
<i>ZmATPS</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 20 s
<i>ZmAPR</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 20 s
<i>ZmOASTL1</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 20 s
<i>ZmOASTL2</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 20 s
<i>ZmDES</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 20 s
<i>ZmRBCL</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 20 s
<i>ZmRBCS</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 25 s
<i>Zmpsba</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 24 s
<i>ZmPEPC</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 57 °C for 30 s and 72 °C for 20 s
<i>Zmactin2</i>	10 min at 95 °C and 40 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 20 s

Table S3 Transmission electron microscopy analysis for the effect of NaHS on the numbers of grana stacks and grana lamellae in mesophyll cell chloroplast ultrastructure of maize leaves. Maize seedlings were pre-treated with 100 μM NaHS for 8 d and then grown in a nutrient solution containing 1 μM Fe(III)-EDTA or 50 μM Fe(III)-EDTA for 12 d. Data are presented as means \pm SE. Different letters in line indicate significant differences at $P < 0.05$ from different treatments. -Fe+NaHS: seedlings were pre-treated with 100 μM NaHS and then treated with 1 μM Fe; +Fe: 50 μM Fe; +Fe+NaHS: seedlings were pre-treated with 100 μM NaHS and then treated with 50 μM Fe.

Variables	-Fe	-Fe+NaHS	+Fe	+Fe+NaHS
Grana stacks (number/chloroplast)	18.8 \pm 2.1 a	23.9 \pm 2.0 b	35.4 \pm 5.6 c	51.9 \pm 3.2 d
Grana lamellae (number/grana)	3.6 \pm 1.2 a	6.5 \pm 0.9 b	10.1 \pm 2.1 c	19.2 \pm 2.0 d

Figure S1 Effect of NaHS on biomass of iron-deficient maize plants during the period of growth.

Maize seedlings were pre-treated with 100 μM NaHS for 8 d and then grown in a nutrient solution containing 1 μM Fe(III)-EDTA ($-\text{Fe}$) for 50 d. Data are presented as means \pm SE.

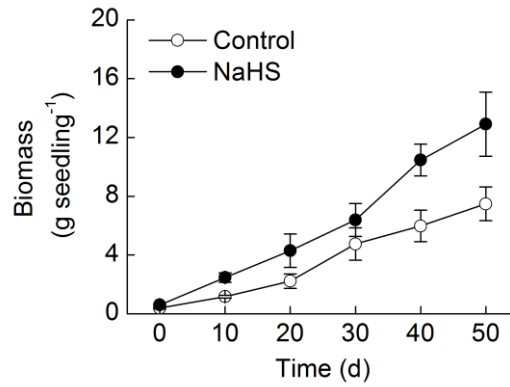


Figure S2 Time-dependent gene expression of *ZmYS1* in iron-deficiency maize roots after NaHS

treatment. Maize seedlings were pre-treated with 100 μM NaHS for 8 d and then grown in a nutrient solution containing 1 μM Fe(III)-EDTA ($-\text{Fe}$) for 12 d. Data are presented as means \pm SE.

