Haem oxygenase-1 is involved in salicylic acid-induced alleviation of oxidative stress due to cadmium stress in *Medicago sativa*. *Weiti Cui, Le Li, Zhaozhou Gao, Honghong Wu, Yanjie Xie, and Wenbiao Shen.*

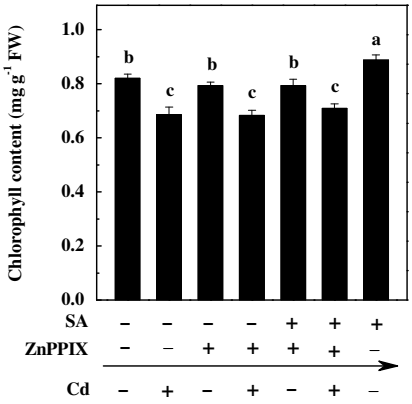
Supplementary Table S1: The sequences of primers for real-time RT-PCR.

Primer name	accession number	Sequences
MsHO1	HM212768	Forward: AACTCTCATTCTCCTCGTTTAGC Reverse: TTCTTTCGCCTGGTCCTTT
APX-1	DQ122791	Forward: TCCTCTTATGCTCCGTTTG Reverse: GTTCCACCCAGTAATCCCA
APX-2	AY054988	Forward: GGAACCATCAAGCACCAAGC Reverse: ACAGCAACAACACCAGCCAAC
ECS	AM407888	Forward: CCTTCGGGTTTGAGCAG Reverse: AGCCTAACCTCGGGAAAT
GS	AM411123	Forward: CTGTCAAATGCCCTTCAATA Reverse: TGTTTCCTCCTCCTTCTCTC
hGS	AM411122	Forward: GTCGCCATCGTTTCTTCCG Reverse: AAGCATTTACGCAGTTTCGC
GR1	AM407889	Forward: TGTGTCATTCGTGGTTGTG Reverse: ACCCGCTATCTTTCCCTC
GR2	AM407890	Forward: TTCCGTTCTCCACAATCTCAT Reverse: TTCACTGTCATATCTCCATCCAA
MDHAR	JN979555	Forward: GTCAAACTAAAGGACGGAAGGGTA Reverse: AGCAACATCGCCAACAGCAT
MSC27	X63872	Forward: AGAATGGAATGTTGTGGGAGG Reverse: GTCATCAACACCCTCATCTTCTC
Actin2	JQ028730	Forward: AAAAGGATGCCTATGTTGGTG Reverse: AAGTGGAGCCTCAGTTAGAAGTA

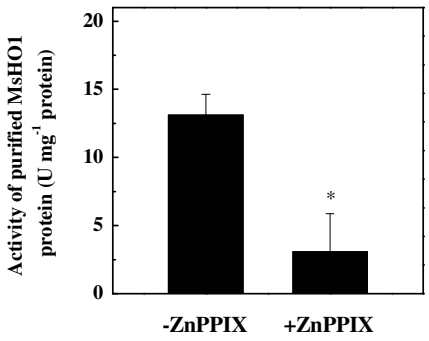
Supplementary Table S2: Reduced and oxidized nicotinamide (NADH and NAD^+, NADPH and $NADP^+$), and the ratio of NADH/NAD^+ and NADPH/$NADP^+$ in alfalfa seedling roots. Five-day-old seedlings were treated with 0 or 50 μM $CdCl_2$ for 24 h with or without 12 h pretreatment with 10 μM SA, 20 μM haemin (H), 100 μM ZnPPIX alone, or their combination treatments. Sample without chemicals was the control (C). Values are means ± SE of three different experiments with at least three replicated for each. Different letters within columns indicate significant differences (P<0.05) according to Duncan's multiple range test.

Treatment	NADH (nmol g^{-1}FW)	NAD^+ (nmol g^{-1}FW)	NADH /NAD^+	NADPH (nmol g^{-1}FW)	$NADP^+$ (nmol g^{-1}FW)	NADPH /$NADP^+$
C → C	7.57±0.61 b	28.85±1.08 e	0.26	7.52±0.33 a	13.54±0.56 d	0.56
SA → C	8.12±0.98 ab	26.17±3.45 e	0.31	7.56±0.38 a	14.68±0.94 d	0.51
C → Cd	9.76±1.74 a	57.73±3.99 ab	0.17	5.63±0.88 cd	23.01±1.56 ab	0.24
SA → Cd	9.35±1.88 ab	41.44±6.01 d	0.23	6.47±0.92 bc	19.51±0.86 c	0.33
SA+ZnPPIX → Cd	8.91±1.36 ab	50.37±1.86 bc	0.18	6.12±0.67 bcd	20.27±1.59 c	0.30
H → Cd	9.54±1.03 ab	45.08±7.28 cd	0.21	6.69±0.68 ab	20.16±2.41 c	0.33
H+ZnPPIX → Cd	8.57±0.44 ab	50.24±7.36 bc	0.17	6.08±0.16 bcd	20.93±2.18 bc	0.29
ZnPPIX → Cd	9.31±1.58 ab	63.41±6.28 a	0.15	5.31±0.66 d	24.18±0.88 a	0.22

Supplementary Figure S1: Effects of SA and ZnPPIX on the chlorophyll content of alfalfa seedling leaves upon Cd stress. 5-day-old seedlings were pretreated with or without 10 μM SA, 100 μM ZnPPIX alone, or their combination treatments for 12 h, then exposed to 0 or 50 μM $CdCl_2$ for another 24 h. Values are means ± SE of three independent experiments with at least three replicates for each. Bars with different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

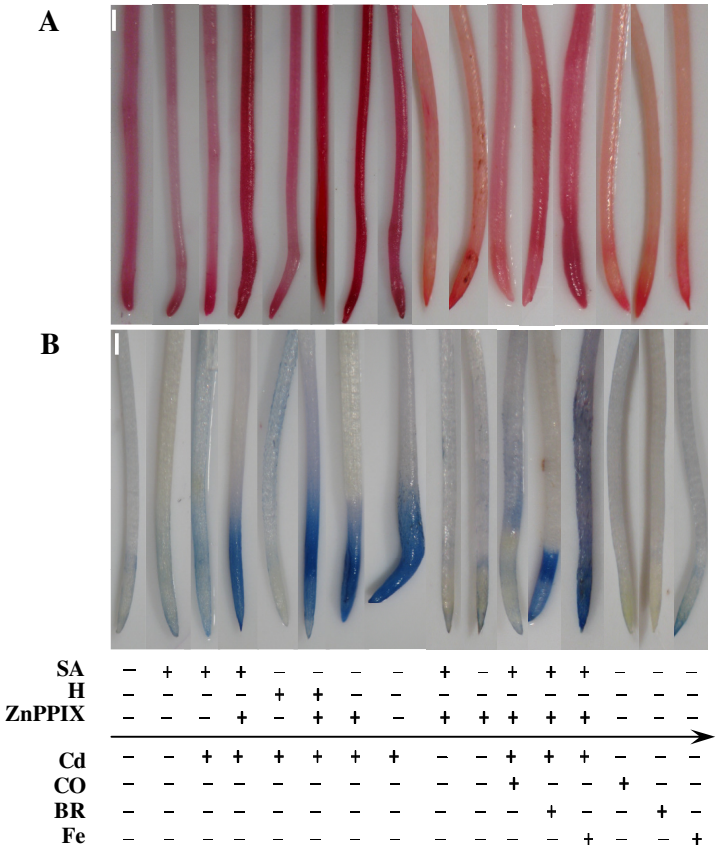


Supplementary Figure S2: Effect of ZnPPIX on the activity of the purified MsHO1 protein. Prokaryotic expression of recombinant MsHO1 protein was induced with IPTG and purified by Ni-affinity chromatography (Fu *et al.*, 2011). The activity of 100-fold dilution of purified protein was detected when incubated with (+ZnPPIX) or without 100 μM ZnPPIX (-ZnPPIX). Values are means ± SE of three independent experiments with at least three replicates for each. Bar with asterisk was significantly different in comparison with the ZnPPIX-free treatment at $P<0.05$ (*t* test).



Supplementary Figure S3: Effects of SA, haemin, ZnPPIX, CO, bilirubin (BR) and Fe (II) citrate (Fe) treatments on the $CdCl_2$-induced lipid peroxidation (A) and the loss of plasma membrane integrity (B) in the root tips of alfalfa.

Five-day-old seedlings were treated with or without 10 μM SA, 20 μM haemin (H), 100 μM ZnPPIX alone, or the combination treatments for 12 h, and then exposed to 50 μM $CdCl_2$, 50% saturation of CO aqueous solution (CO), 20 μM bilirubin (BR), 20 μM Fe (II) citrate (Fe) alone, or the combination treatments for another 24 h. Afterwards, the roots were stained with Schiff's reagent (A) or Evans blue (B), and immediately photographed under a light microscope. Sample without chemicals was the control. Scale bar = 1 mm.



Supplementary Figure S4: Confocal images of ROS production in root tips of alfalfa.

Five-day-old seedlings were treated with or without 10 μM SA, 20 μM haemin (H), 100 μM ZnPPIX alone, or the combination treatments for 12 h, and then exposed to 0 or 50 μM $CdCl_2$ for another 24 h. Sample without chemicals was the control (C). The distribution of ROS in root tips was detected by fluorescence probe H_2DCFDA after different treatments under fluorescence and bright field microscopy (TCS-SP2 confocal laser scanning microscope; Leica Lasertechnik GmbH, A). Lines 1-8 represent different treatments as follows: (1) C→C; (2) SA→C; (3) SA→Cd; (4) SA+ZnPPIX→Cd; (5) H→Cd; (6) H+ZnPPIX→Cd; (7) ZnPPIX→Cd; and (8) C→Cd. Scale bar = 300 μm. Mean relative H_2DCFDA fluorescence densities corresponding to (A) were given in (B), taking control (C) as 100%. Values are means ± SE of three independent experiments. Bars with different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.