Hydrogen sulphide 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

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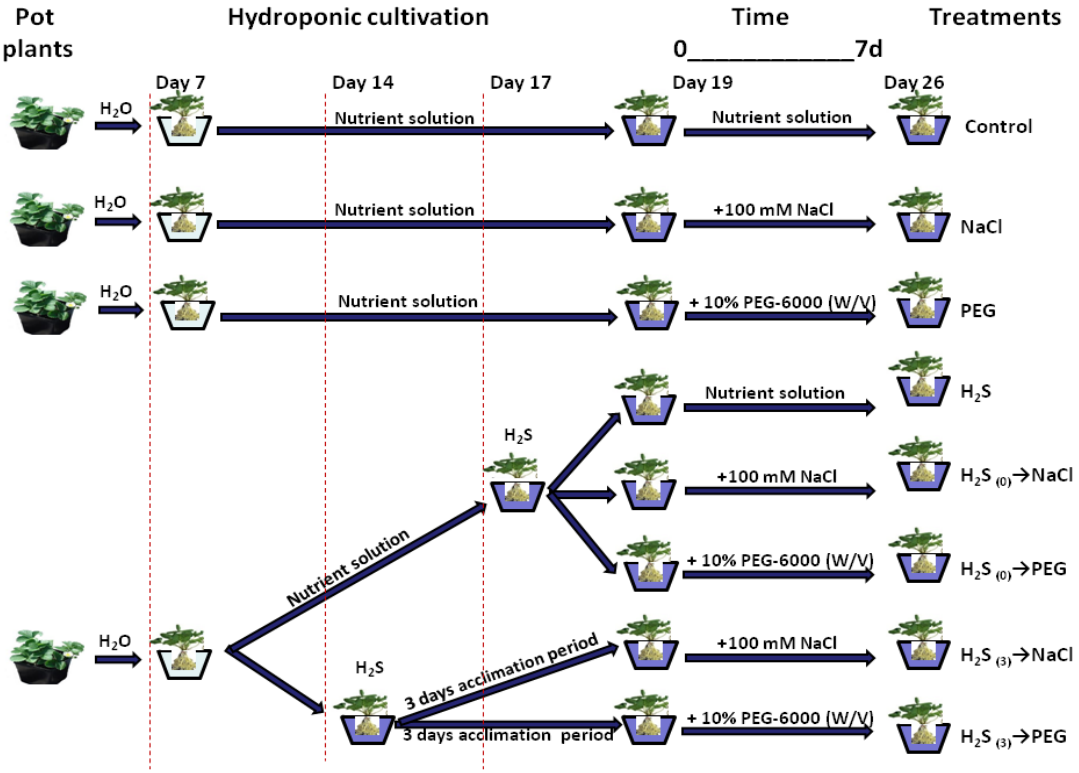
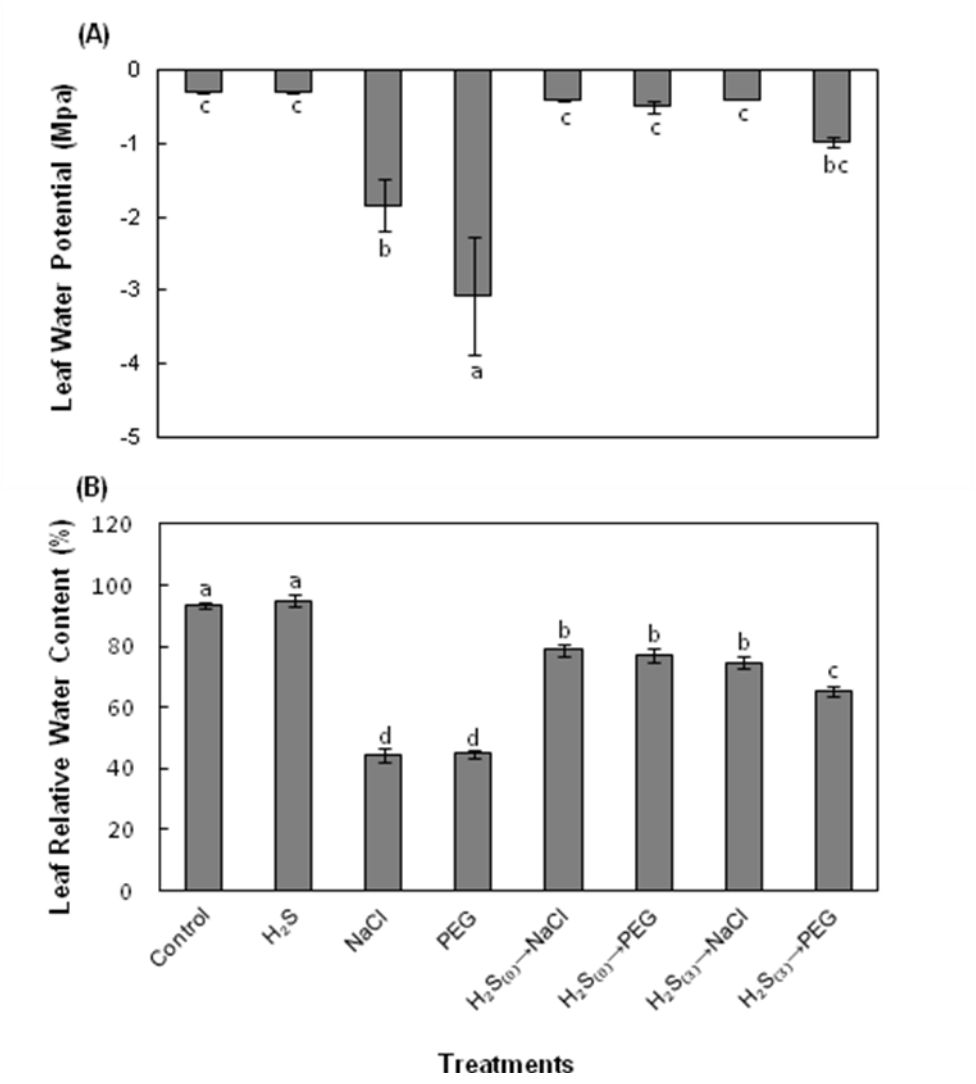
Supplementary Data



Supplementary Figure S1. Schematic depiction of experimental treatments. Control (pretreated with H_2O, no acclimation period, not stressed), H_2S (pretreated with H_2S, no acclimation period, not stressed), NaCl (pretreated with H_2O, no acclimation period, 100 mM NaCl stressed), PEG (pretreated with H_2O, no acclimation period, 10% (w/v) PEG-6000 stressed), $H_2S_{(0)}$→NaCl (pretreated with H_2S, no acclimation period, 100 mM NaCl stressed), $H_2S_{(0)}$→PEG (pretreated with H_2S, no acclimation period, 10% (w/v) PEG-6000 stressed), $H_2S_{(3)}$→NaCl (pretreated with H_2S, 3 days acclimation period, 100 mM NaCl stressed), $H_2S_{(3)}$→PEG (pretreated with H_2S, 3 days acclimation period, 10% (w/v) PEG-6000 stressed).



Supplementary Figure S2. Effects of H_2S donor NaHS (100 µM) on leaf water potential **(A)** and leaf relative water content **(B)** on strawberry plants exposed either to 100 mM NaCl or 10% (w/v) PEG-6000, for 7 days. Treatment acronyms are described in Supplementary Fig. 1 caption. Data are means ± SE of 3 replications. Bars with different letters are significantly different at $p < 0.05$.

Supplementary Table S1. Oligonucleotides used as primers for real-time RT-PCR.

Gene	Primer	Primer sequence	Reference	Ta (°C)
18S	For	5-ACC GTA GTA ATT CTA GAG CT-3	Bustamante et al. (2006)	46
	Rev	5-CCA CTA TCC TAC CAT CGA AA-3		
GS	For	5′-GGG ACG AAA AAG GTT CAA CA-3′	DY668932*	56
	Rev	5′-GCA TCA GGA TGT AAG CAG CA-3′		
GCS	For	5′-ACA GGC TCG TCC TAC TGC AT-3′	DY672795*	56
	Rev	5′-TCC TAC ACC TGC ATT GTC CA-3′		
GDH	For	5′-CAA GGT CGA GCT TCG AGA AC-3′	TA10972_57918*	56
	Rev	5′-CAA GCA CCT TCT CCG ACA AT-3′		
GR	For	5′-AGC ATG ACT GGA GCA CAT TG-3′	TA2829_57918*	56
	Rev	5′-AGG CAA ATC AAG AGC AGC AT-3′		
CAT	For	5′-CAC CTG TCA TTG TGC GTT TC-3′	TA2053_57918*	56
	Rev	5-CTT TCT GGA TGG TGG GAA AA-3′		
cAPX	For	5′-CAC AAG GAA CGG TCT GGA TT-3′	TA478_3747*	56
	Rev	5′-CGC AGC GTA TTT CTC AAC AA-3′		
MnSOD	For	5′-TGT GGC TGG CTT TAG ACA AA-3′	CO381280*	56
	Rev	5′-CTT CCA ATT GAT GAC CTT CCA-3′		
NR	For	5′-AGG GAA ACA CCC CTT CAA CT-3′	TA2111_57918*	56
	Rev	5′-TGC TTC ACC ATG TTC TGC TC-3′		
DREB	For	5′-CGG AGT TGG TTT TCC AAG AA-3′	TA10928_57918*	56
	Rev	5′-TCC GGG TAC TCG TTC AAA TC-3′		
SOS2-like	For	5′-CCG TGC AAG AAG AAG AGG TC-3′	DY669538*	56
	Rev	5′-ATT TGA CCT TGG CGA AAT TG-3′		
SOS3-like	For	5′-TTC CCA ACC CAT TAA ACC AA-3′	DY670450*	56
	Rev	5′-GTT TGT GAA GAG AGG GAG AC-3′		
SOS4	For	5′-TGG AGA ACA GCT TTG GGA AT-3′	DY672501*	56
	Rev	5′-GCT CTGGAG GGA CAT ACA GC-3′		

* *Fragaria* EST sequence Accession no.

Supplementary Table S2. Effects of H_2S donor NaHS on the relative mRNA expression (fold change) of enzymatic antioxidants and of proteins involved in RNS biosynthesis, redox homeostasis, SOS pathway and transcription regulation, in leaves of strawberry plants under non-stress, salt and PEG stress conditions compared with controls, determined by qRT-PCR. Following root pre-treatment with H_2S 3 days before stress imposition or until application of stress factor, the experimental plants were grown in absence or presence of 100 mM NaCl or 10% (w/v) PEG-6000 for 7 days as described in Suppl. Figure 1. Tissues were sampled immediately after H_2S pre-treatment (Day 0) and 7 days after pre-treatment (Day 7). Values in bold letters denote statistically different values according to pairwise fixed reallocation randomisation test ($P < 0.05$). Values in red represent significantly suppressed samples, while green boxes represent significantly induced samples. *18S* rRNA was used as a housekeeping gene control.

		Gene of interest relative expression ratio (Treated/Control)											
Treatment/ Day		***cAPX***	***CAT***	***GR***	***MnSOD***	***GDH***	***GCS***	***GS***	***NR***	***SOS2-like***	***SOS3-like***	***SOS4***	***DREB***
H_2S	0	1,028	1,035	1,617	-1,173	1,040	1,441	-1,388	1,146	1,151	-1,540	1,857	1,505
NaCl	0	-1,329	-1,945	1,457	-1,272	-1,122	-1,289	-1,254	1,220	-1,693	-1,254	1,028	1,009
PEG	0	-1,427	-1,350	1,533	-1,146	-1,437	1,251	1,544	1,141	-1,005	-1,862	-1,411	1,332
$H_2S_{(0)}$→NaCl	0	1,357	-1,512	-1,057	1,301	-1,248	1,012	-1,269	-1,338	-1,761	-2,104	1,551	1,566
$H_2S_{(0)}$→PEG	0	1,189	1,062	1,130	2,028	-1,335	1,945	-1,125	-1,347	-1,537	1,084	-1,120	1,745
$H_2S_{(3)}$→NaCl	0	-1,576	-1,566	-1,016	-1,019	-1,295	-1,803	-1,173	-1,173	-1,495	-1,666	1,298	1,225
$H_2S_{(3)}$→PEG	0	1,040	1,350	1,316	-1,057	-1,014	2,229	1,094	-1,102	-1,151	-1,237	-1,613	-1,366
H_2S	7	1,686	1,316	-1,437	1,295	1,234	1,591	1,045	1,357	1,072	1,050	-1,628	1,434
NaCl	7	**-14,487**	**-10,803**	**-5,028**	**-1,910**	**-7,243**	**-2,144**	**-12,641**	**-6,483**	**-8,130**	**-2,474**	**-24,590**	**-2,189**
PEG	7	**-15,102**	**-4,408**	**-3,873**	**-3,356**	**-3,212**	-1,537	**-4,757**	**-4,779**	**-3,109**	**-22,838**	**-26,052**	**-2,508**
$H_2S_{(0)}$→NaCl	7	-1,610	-1,210	-2,080	**3,918**	-1,498	1,431	-2,047	-2,109	-2,019	**4,056**	-1,237	-1,509
$H_2S_{(0)}$→PEG	7	-1,167	-2,440	-2,214	1,173	-2,037	1,033	-1,548	**-2,751**	-2,245	1,217	**-6,574**	**-2,134**
$H_2S_{(3)}$→NaCl	7	**-4,779**	**-3,779**	**-2,770**	**-4,387**	**-3,180**	**-2,515**	**-3,828**	**-3,837**	-2,126	-1,427	-1,504	-1,982
$H_2S_{(3)}$→PEG	7	**-2,09**	**-8,574**	**-4,735**	**-2,881**	**-2,764**	**-1,769**	**-3,294**	**-2,09**	**-2,707**	**-2,451**	**-9,514**	-2,028