

Supplementary material

H₂S Enhanced the Tolerance of *Malus hupehensis* to Alkaline Salt Stress through the Expression of Genes Related to Sulfur-Containing Compounds and the Cell Wall in Roots

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Table S1 Sequence of qRT-PCR primers for deferentially expressed genes

Gene ID	Gene description	Forward primer	Reverse Primer
LOC103438253	low affinity sulfate transporter 3	AATCCAATCTTCTTCCTC	ATTAGCATATCCAATACTCT
LOC103423770	uncharacterized LOC103423770	AAGTCTAGTAGTAGTAGTAGT	CTAAGGAATATCAGGATGG
LOC103423808	protein ALTERED XYLOGLUCAN 4-like	CAATATCATCACTCATCATC	AGGAGAGGAAGAATAAGA
LOC103443393	hydroxyproline O-galactosyltransferase HPGT1-like	GAACTTGATAGGATTACTG	CGCTGATAACTTCTTATG
LOC103454744	probable phospholipid hydroperoxide glutathione peroxidase	GATAAGGTGGATGTGAAT	CTAAGAGGAGAAGTTGTAG
LOC103454388	thioredoxin reductase NTRB-like	CTGAGCCTGATGAGAAGAAG	GTGGAAGCGGAAGAAGAC
LOC103449875	hexokinase-1-like	ATTATACGAGCATTACACTGAAT	CCAGAGCCATCATTAGAGT
LOC103424166	basic leucine zipper 9-like	CGTCTCACCAACCATTAC	ACAGTATCGCTCATCATCA
LOC103435863	galacturonosyltransferase 8	TGTCCATATTGAACCACTTG	AACCACTATATCGTCATCCA
LOC103404371	ferredoxin-thioredoxin reductase catalytic chain, chloroplastic	GGAGCCGTCAGATAAGTC	CCTTGATAACAACAGAAGTAACT

Table S2 Summary of clean reads and genes mapped to the reference genome and gene from H₂S and alkaline salt stress roots of *M. hupehensis*

Sample	Total Clean Reads genome	Total Mapping(%) genome	Uniquely Mapping(%) genome	Total Clean Reads gene	Total Mapping(%) gene	Uniquely Mapping(%) gene
Control-1	40.84 M	72.86 %	34.84 %	40.84 M	68.28 %	29.21 %
Control-2	38.23 M	72.97 %	34.85 %	38.23 M	68.69 %	29.13 %
Control-3	39.38 M	73.21 %	34.91 %	39.38 M	69.28 %	29.19 %
H ₂ S-1	42.2 M	73.24 %	35.1 %	42.2 M	68.87 %	28.77 %
H ₂ S-2	41.74 M	73.8 %	35.25 %	41.74 M	69.55 %	29.04 %
H ₂ S-3	41.85 M	72.97 %	34.82 %	41.85 M	68.22 %	28.57 %
H ₂ S+AS-1	41.82 M	73.48 %	34.12 %	41.82 M	68.27 %	29.91 %
H ₂ S+AS-2	42.71 M	73.85 %	34.44 %	42.71 M	68.89 %	29.41 %
H ₂ S+AS-3	43.78 M	72.89 %	34.21 %	43.78 M	67.67 %	29.03 %
AS-1	42.63 M	72.23 %	33.37 %	42.63 M	67.03 %	28.8 %
AS-2	42.12 M	72.31 %	33.89 %	42.12 M	66.94 %	28.66 %
AS-3	37.82 M	72.85 %	33.8 %	37.82 M	67.45 %	29.49 %

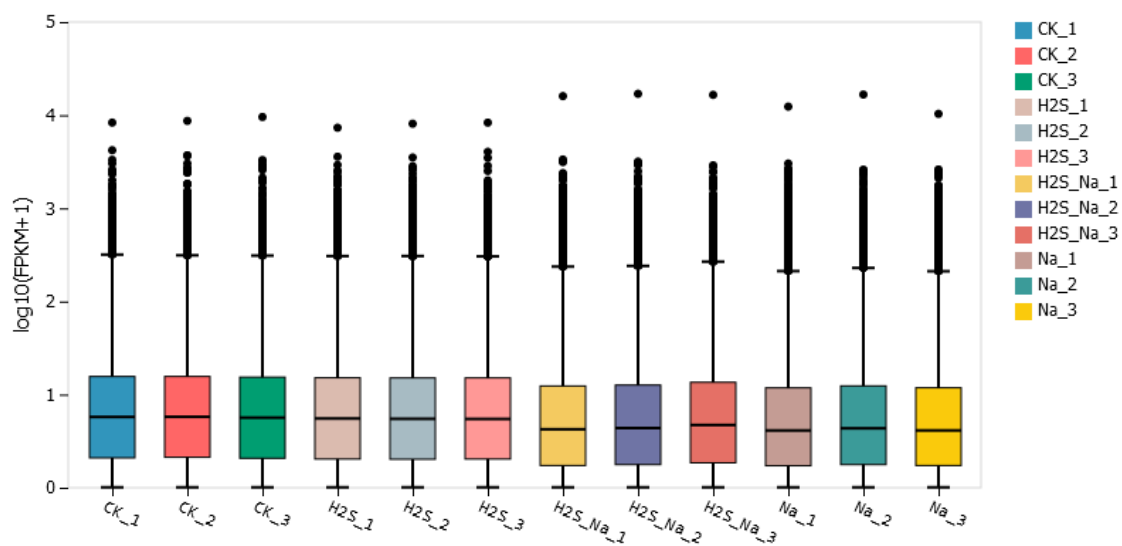


Figure S1 The box plot shows the distribution of gene expression level of each sample, and the dispersion of data distribution can be observed.

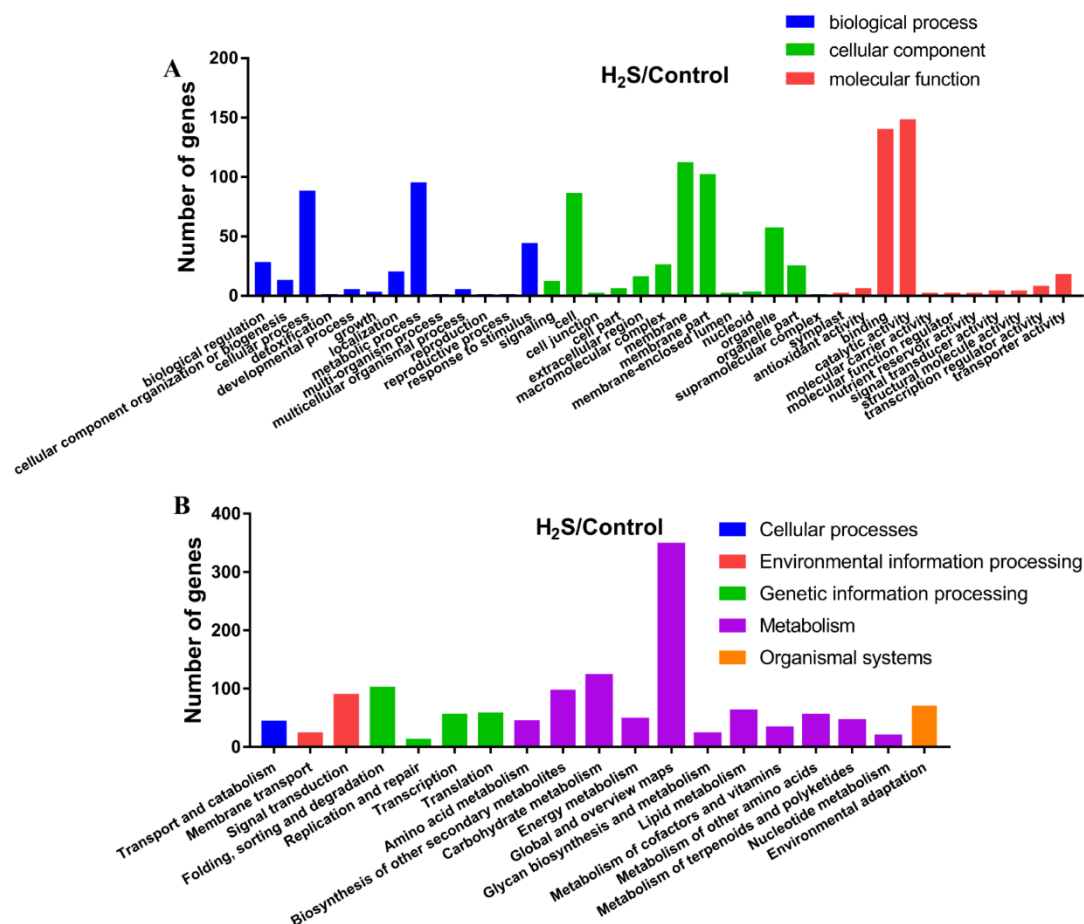


Figure S2 Gene ontology and KEGG pathway classification of differentially expressed genes in H₂S compared with control group. GO classification of DEGs. Blue, green and red represent three GO ontologies: biological process, cellular component, molecular function and, respectively (A). KEGG pathway classification of DEGs. Blue, red, green, purple, and orange represent the different KEGG pathway: cellular processes, environmental information processes, genetic information processes, metabolism, and organism systems (B).

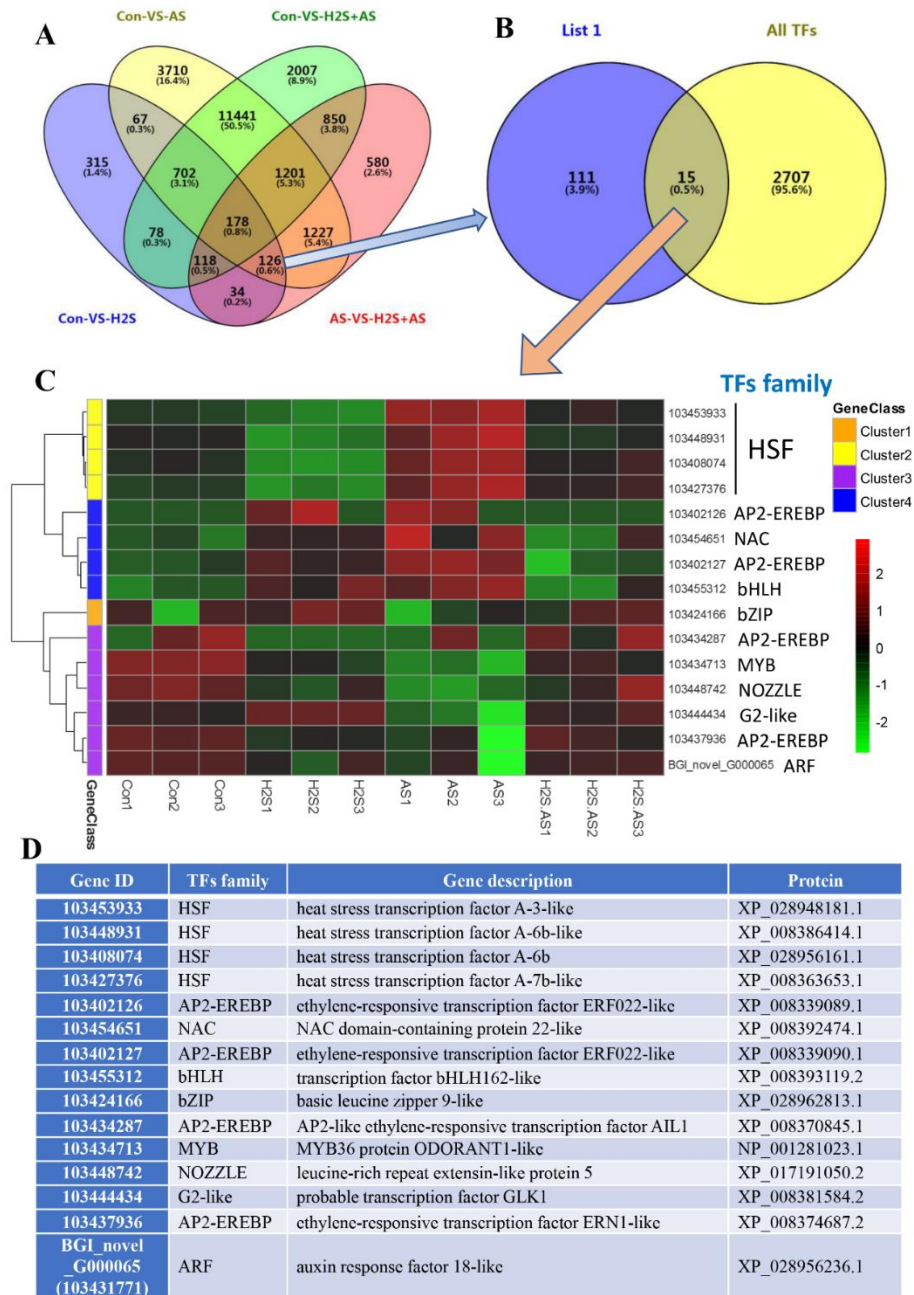


Figure S3 HSF family transcription factors play an important role in the process of H_2S mediated alkaline salt stress relief. Venn of the number of DEGs in different comparison groups (A). Identification of differentially expressed key transcription factors (B). The expression patterns of differentially expressed TFs were analyzed by heatmap (C). The information of key TFs may play an important role in the process of H_2S mediated alkaline salt stress relief (D).