Electronic Supplementary Information for

Characterizing the Uptake, Accumulation and Toxicity of Silver Sulfide Nanoparticles in Plants†

Peng Wang,*[ab] Enzo Lombi, *[c] Shengkai Sun,[a] Kirk G. Scheckel,[d] Anzhela Malysheva,[ce] Brigid A. McKenna,[b] Neal W. Menzies,[b] Fang-Jie Zhao,[a] and Peter M. Kopittke[b]

[a] *Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, Jiangsu, 210095, China*

[b] *The University of Queensland, School of Agriculture and Food Sciences, St. Lucia, Queensland, 4072, Australia*

[c] *University of South Australia, Future Industries Institute, Mawson Lakes, South Australia, 5095, Australia*

[d] *United States Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio, 45268, USA*

[e] *University of Copenhagen, Department of Plant and Environmental Sciences, Copenhagen, 1165, Denmark*

Summary
13 pages, including 2 tables and 9 figures.

Table S1. Primer sets used in the present study.

Gene	Name	Primers
Cucumber aquaporins	CsaActin	5′-GCTGGATTCTGGTGATGGTGTGAG-3′ and 5′-TTCGGCAGTGGTGGTGAACATG-3′
	CsaPIP1;1	5′-TCCGCCACCGATGCTAAGAGAA-3′ and 5′-AAGCGTGTTGCCTGTTGTAGATGAT-3′
	CsaPIP1;2	5′-ACCGTCTTCTCCGCCACTGAT-3′ and 5′-ATCCCACGCTTTGTCCTTGTTGAA-3′
	CsaPIP2.1	5′-TTGCGGCTGTGGCTTGGTTAAG-3′ and 5′-TCTCTGGCGTTTCTCTTGGGATCA-3′
	CsaPIP2.2	5′-TACTGTCTTCTCCGCCACTGATCC-3′ and 5′-TGATTGTTGATGATGACGGCAGGAC-3′
	CsaPIP2.3	5′-TGTGGCTGTGCATTGGTGAAGG-3′ and 5′-TCTCTTGGGATCGGTAGCGGAAA-3′
	CsaPIP2.5	5′-TCTTCTCCGCCACCGATTCCAA-3′ and 5′-GCCCAACCCAGAATATCCAATGGT-3′
	CsaPIP2.6	5′-GGCTCTTGGTGCTGAGATTATTGGA-3′ and 5′-CCTGTGATGGGAATGGTTGCTAGAT-3′
Wheat aquaporins	TaActin	5′-TCCTGGAATTGCTGATCGCATGAG-3′ and 5′-AGACTCATCGTACTCCGCCTTGG-3′
	TaPIP1	5′-CGCCGTCATCTACAACAAGGACAA-3′ and 5′-GCCATTGCTTCAGTCTTGCATACAG-3′
	TaPIP2	5′-AAGAAGGCCGCCTCCAGCAA-3′ and 5′-GGAAGAAACAGCAGCAAGCAAACAT-3′
	TaPIP3	5′-GCGGCGGTAGTAGATGTTGTTCTT-3′ and 5′-GCTGGCTGCTACTTGTCGCTATT-3′
	TaAQP1	5′-GACCAAGTCCTAAGCCGCTCCT-3′ and 5′-GTCTGGGTCCAGGTTCAGGTGTAT-3′
	TaAQP2	5′-CTGCTGTGGTCTACAACAACGAGAA-3′ and 5′-CCGTGCTTCCGATCATCCATACTG-3′
	TaAQP3	5′-GAGCTGCTGTGATCTACAACAACGA-3′ and 5′-CGATCTAGCGACTGCCGAAGGA-3′
	TaAQP4	5′-GGCTTCGCCGTCTTCATGGT-3′ and 5′-CCCGGAGGATGTACTGGTGGTA-3′
Cucumber ethylene signalling pathway related genes	Csa6M318160	5′-AGAGCATACGATGAAGCTGCCAAG-3′ and 5′-ACTCCTCGCTGTCACACGAACTA-3′
	Csa4M001970	5′-GGCTCTGGGTCGGAGGATTCTTAT-3′ and 5′-CACCTGTTCTTCTGCTGGATTCGT-3′
	Csa3M164580	5′-TGGCACCTACAACACTCCTGAAGA-3′ and 5′-GGCGGTGAGGGTGGTAGAGATAA-3′
	Csa3M878200	5′-TGCTCGATCACGGATTCAGGAATTG-3′ and 5′-GCTGGATGTTGAGACCCACAAGAG-3′
	Csa7M405830	5′-AGGAACGACAGCCGTGTTCATTG-3′ and

	Csa2M070880	5′-CGGGAACTGGAGAAGGAAACTTGAC-3′ 5′-GCACTCCTGGTGTTGATGGTTATGA-3′ and 5′-CCGACGCTCTATAAGTTCCGACAA-3′
Cucumber miraculin-like protein (MLP) genes	Csa1M043200 Csa2M021500	5′-GCGAGTACGATCTGCGTGACATC-3′ and 5′-AAGCCAAAGCCCTTTCTCCATTCTT-3′ 5′-TGCAACTGAGTCTGATACCGGAAGA-3′ and 5′-AAGCCACCAGCCTCACTCCATT-3′

Table S2. The percentage speciation of Ag in various tissues of cucumber and wheat plants as calculated using linear combination fitting (LCF) of the K-edge XANES spectra.

Treatment	Metallic Ag	Ag_2S	Ag-glutathione	R-factor
Cucumber root				
$AgNO_3$	10 (1.2)		90 (1.2)	0.00015
Ag_2S-NPs		94 (0.9)	6 (0.9)	0.00008
Cucumber leaf				
$AgNO_3$	6 (1.8)		94 (1.8)	0.00029
Ag_2S-NPs		93 (1.1)	7 (0.7)	0.00014
Wheat root				
$AgNO_3$	12 (0.8)		88 (0.8)	0.00006
Ag_2S-NPs		99 (0.3)	1 (0.3)	0.00006
Wheat shoot				
$AgNO_3$	2 (2)		98 (2)	0.00006
Ag_2S-NPs		91 (1.4)	9 (1.4)	0.00041

The values in brackets show the percentage variation in the calculated values. The goodness of fit is indicated by the R-factor. R factor = $\sum i(\text{experimental} - \text{fit})^2/\sum i(\text{experimental})^2$, where the sums are over the data points in the fitting region.

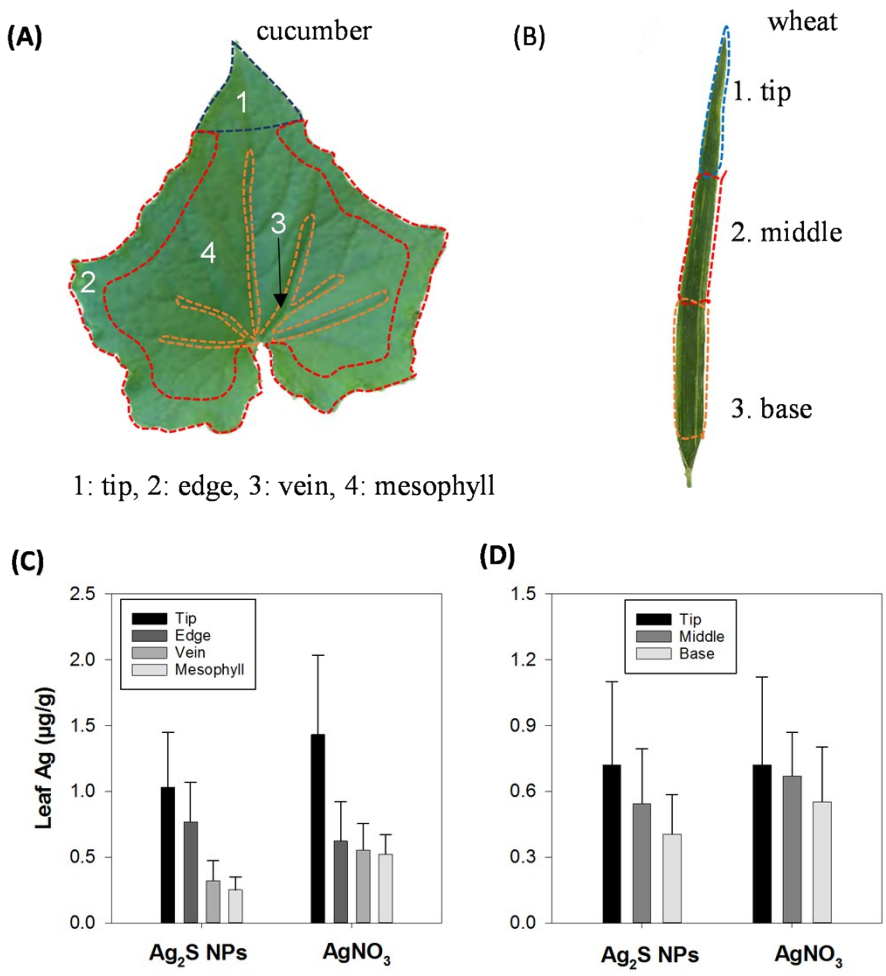

Figure S1. Approximate division of leaves and concentrations of Ag in various fractions of leaves of cucumber (A, C) and wheat (B,D). Cucumber leaves were divided into four fractions: tip, edge, vein, and interveinal mesophyll tissues, while wheat leaves were evenly divided into three fractions: tip, middle, and base.

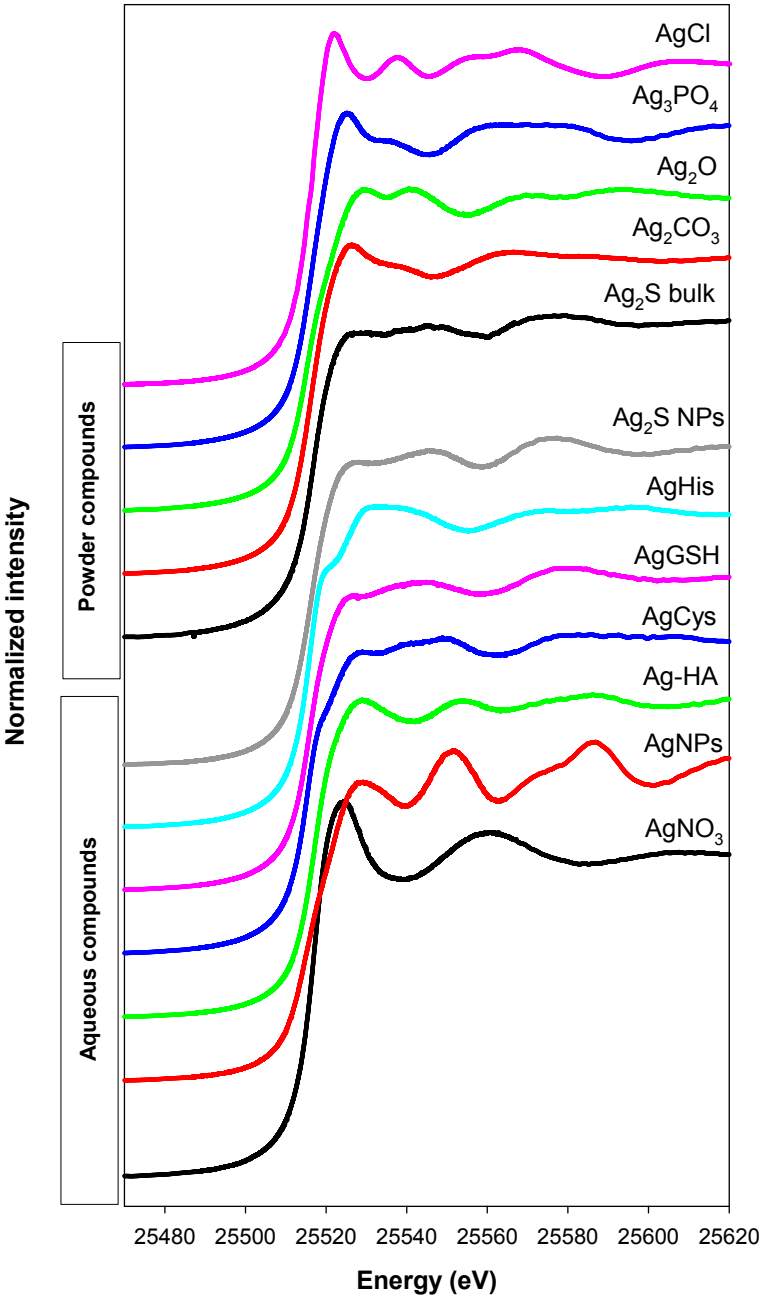

Figure S2. Ag K-edge XANES spectra of various Ag standard compounds.

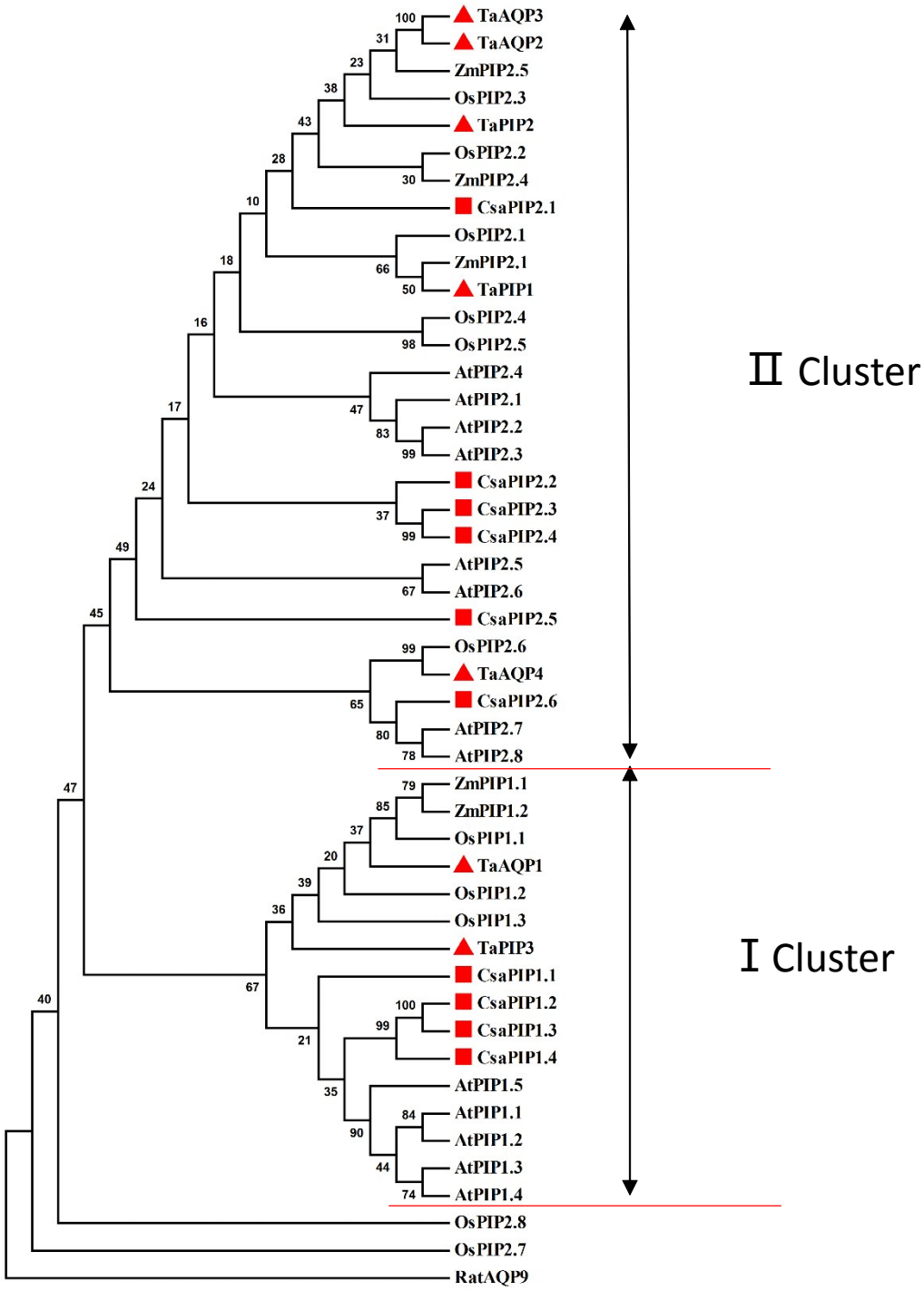

Figure S3. Classification of PIP gene family in cucumber and wheat based on the homology of protein sequences.

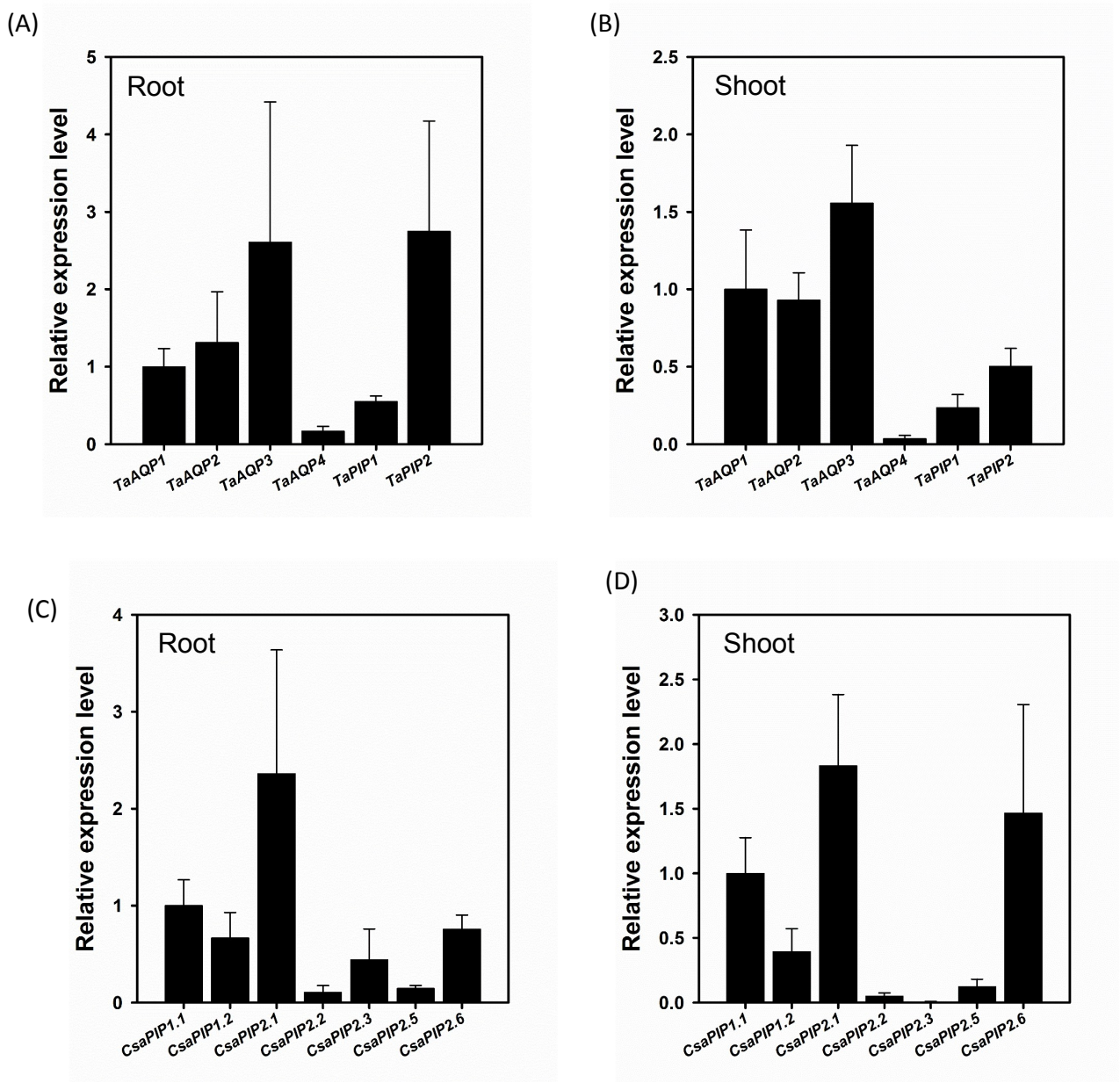

Figure S4. Relative expression of various aquaporin genes in roots (A, C) and shoots (B, D) cucumber (A, B) and wheat (C, D). Data are means ± SD.

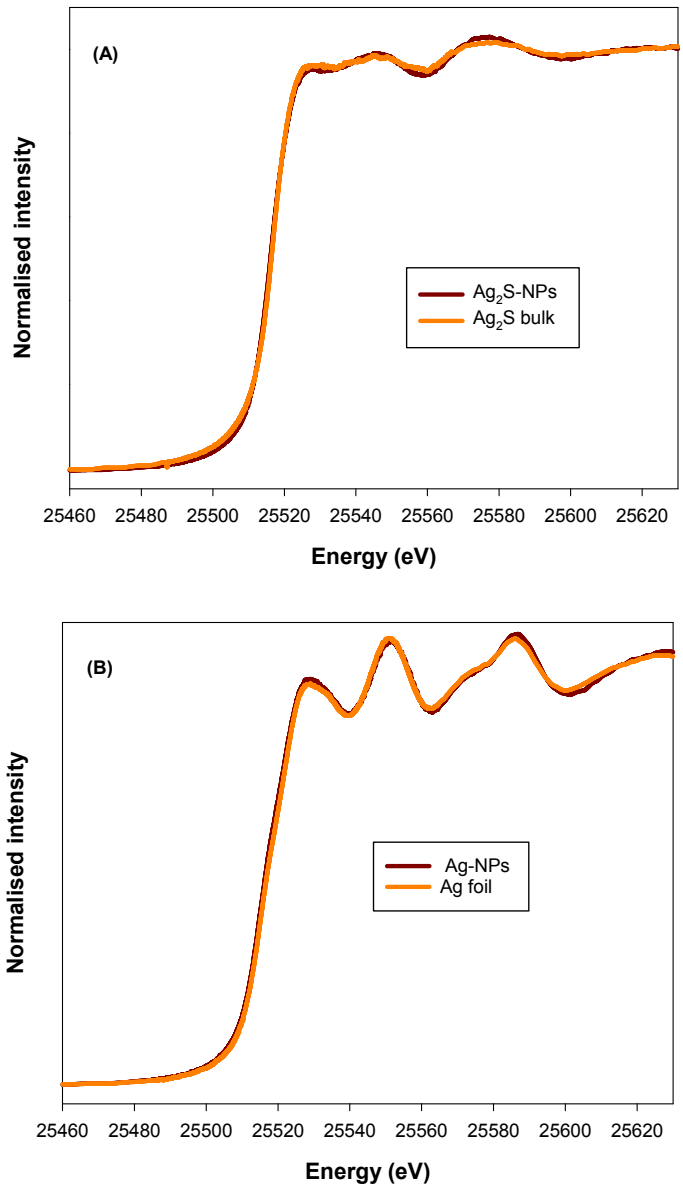

Figure S5. Comparison of XANES spectra of Ag_2S-NPs (A) and Ag-NPs (B) with those of bulk Ag_2S and Ag foil.

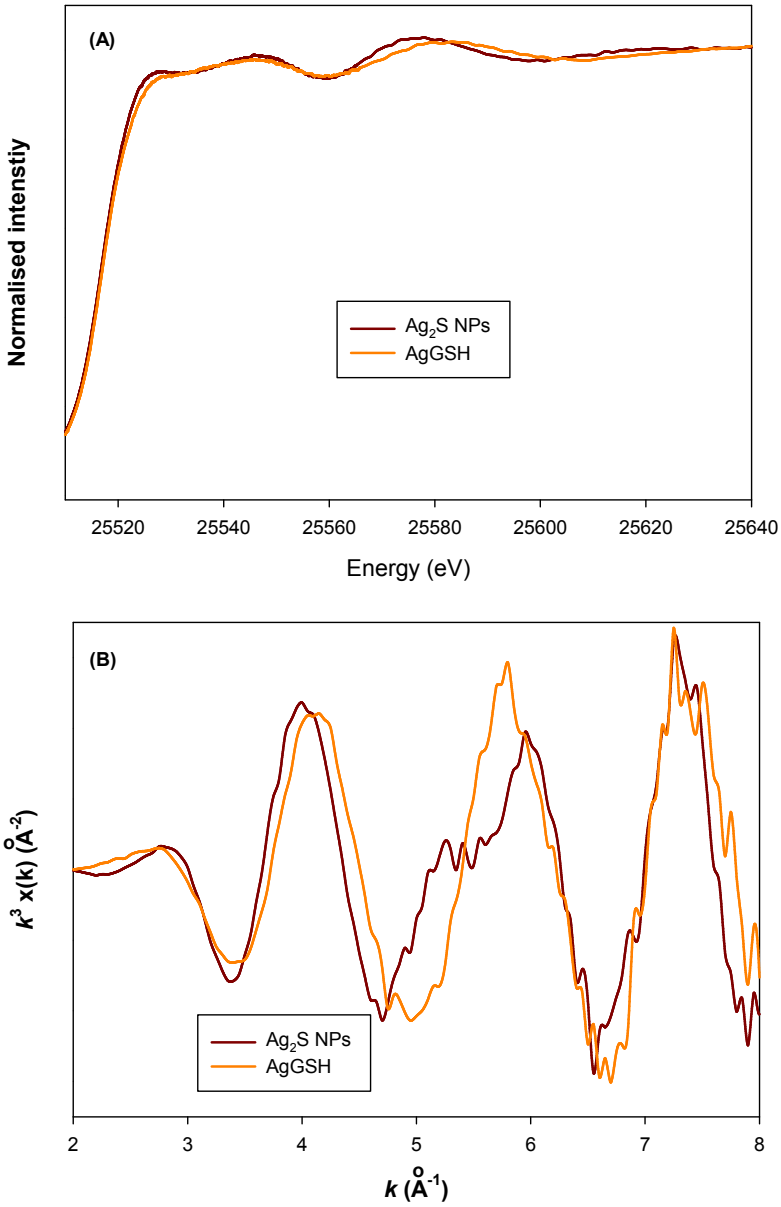

Figure S6. Ag K-edge XANES spectra (A) and k^3-weighted EXAFS spectra (B) of reference Ag_2S-NPs and Ag-glutathione (AgGSH).

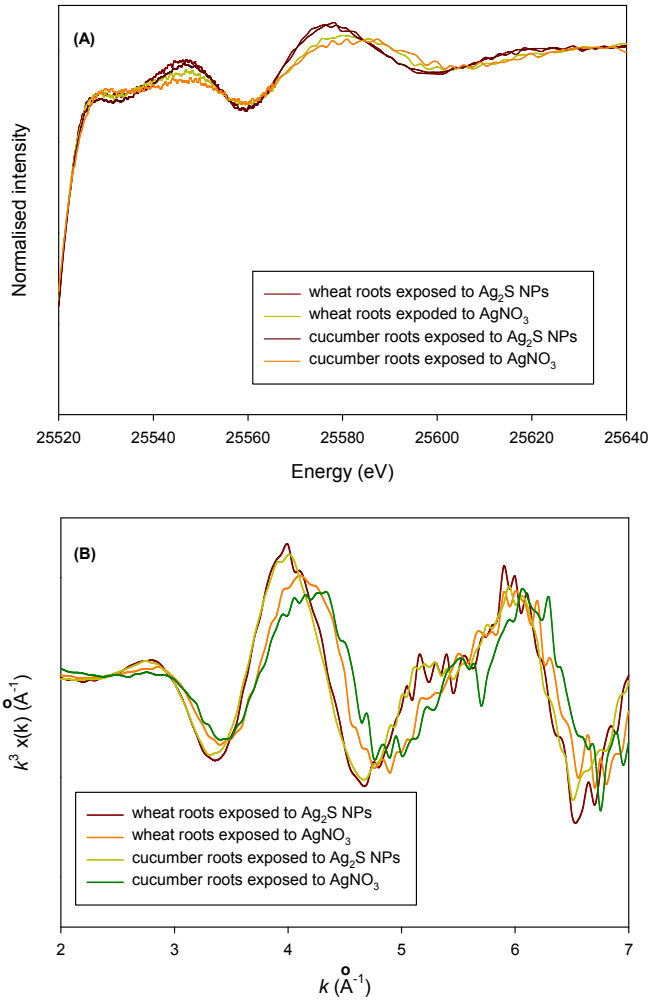

Figure S7. Comparison of the XANES spectra of Ag_2S-NP treated plants and those of $AgNO_3$-treated plants.

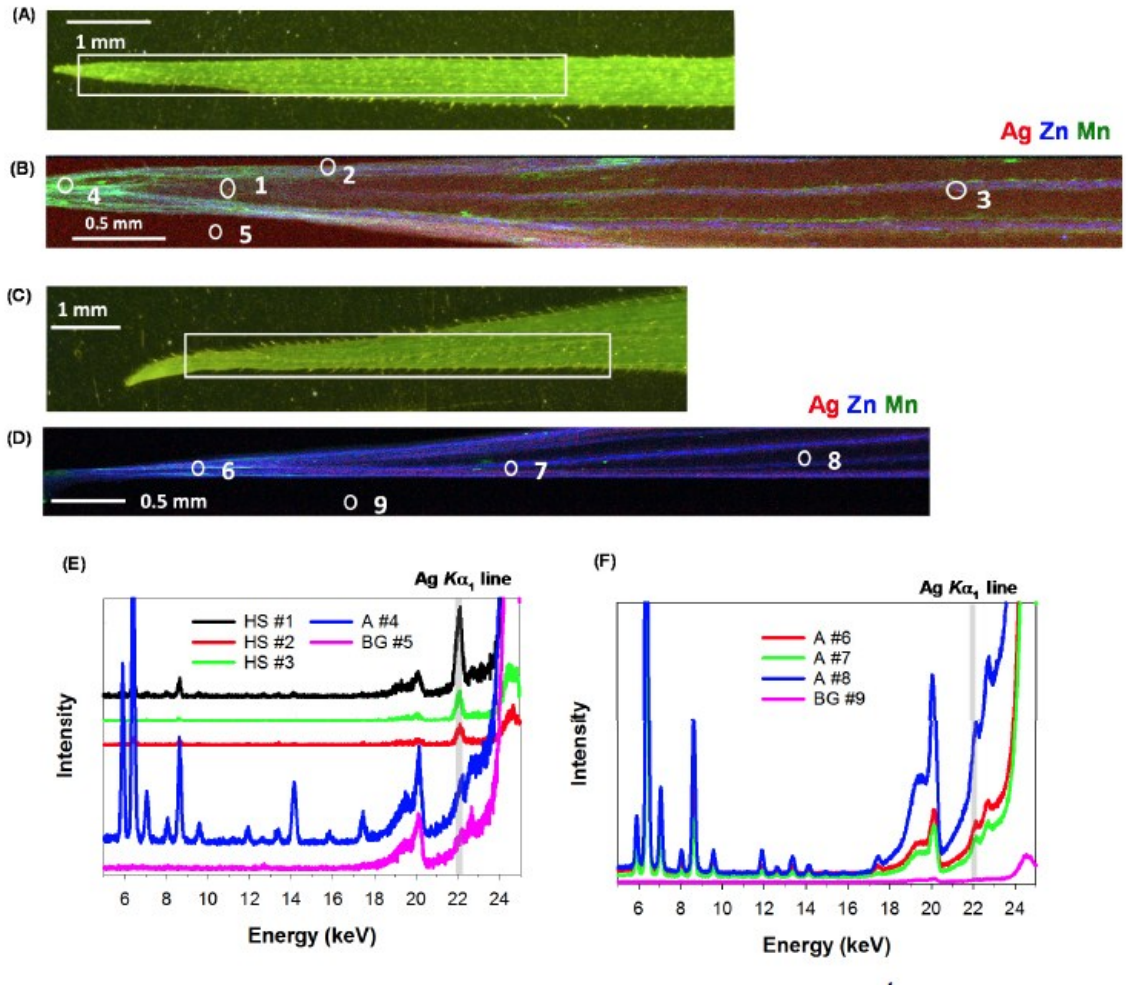

Figure S8. Leaves of wheat grown in nutrient solution containing 10 mg Ag L^{-1} as Ag_2S-NPs or 0.5 mg Ag L^{-1} as $AgNO_3$ for one week. (A, C) Light micrograph after µ-XRF analysis, with the white box indicating the area examined by µ-XRF. (B, D) Tricolor µ-XRF map of Ag (red), Zn (blue), Mn (green) distribution (top panel) of leaves of wheat exposed to Ag_2S-NPs (B) or $AgNO_3$ (D). (E, F) Fluorescent intensity of µ-XRF spectra collected at 27 keV for hotspots (HS #1-3), and selected areas (A #4, #6-8) background (BG #5, #9) as shown in (B) and (D).

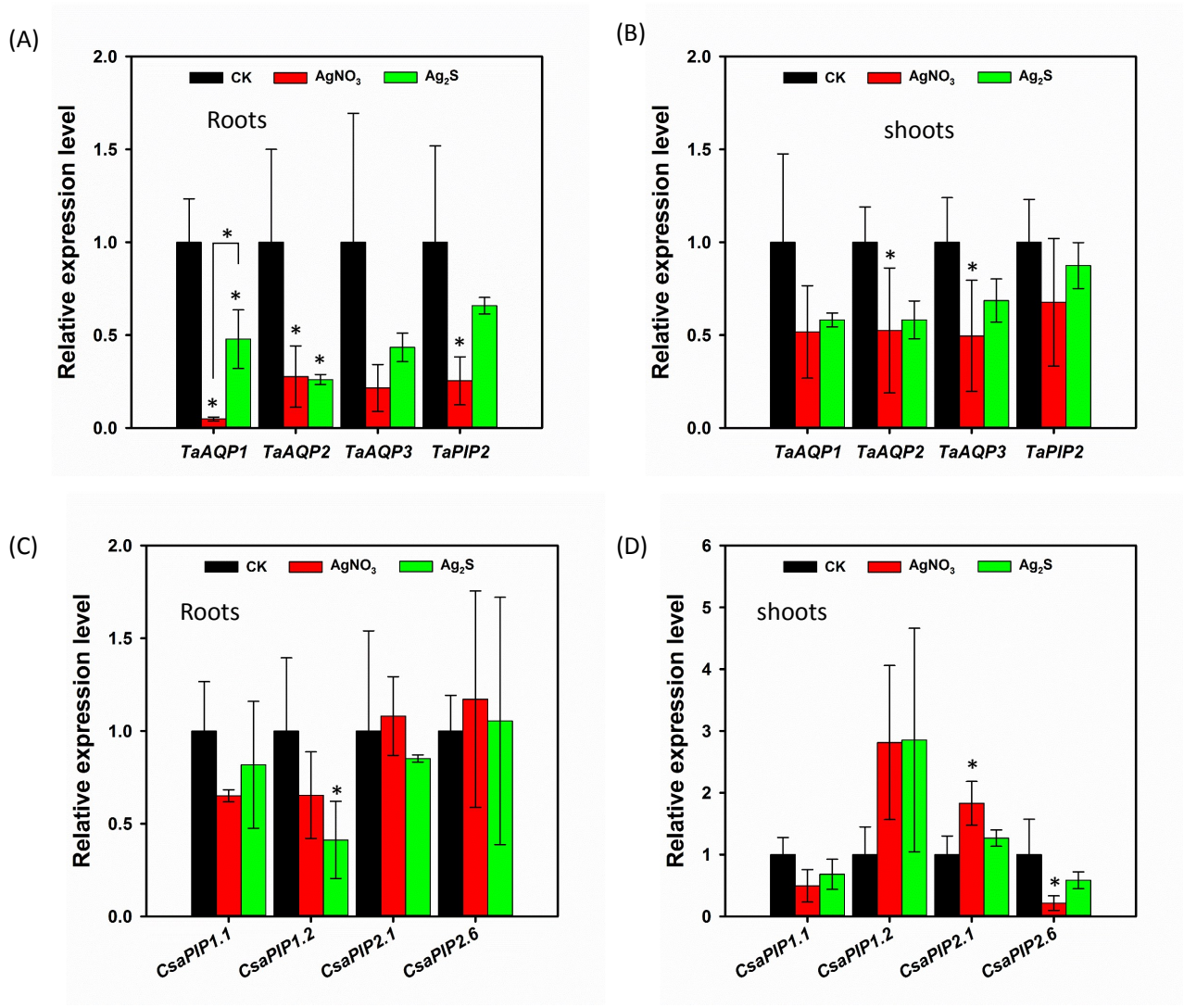

Figure S9. Relative expression of aquaporin genes in roots (A, C) and shoots (B, D) of cucumber (A, B) and wheat (C, D) exposed to Ag_2S-NPs or $AgNO_3$ for one week. Data are means ± SD. * indicates significant difference compared to the control.