

**Figure S1. Drought-tolerant phenotype of the** *ahp4* **mutant plants.** A, Two-week-old *ahp4* and wild-type (WT) plants were transferred from germination medium plates to soil and grown for one additional week. B, Three-week-old plants were subjected to drought for 15 days and plants were photographed three days subsequent to rewatering and after removal of inflorescences. C, Five-week-old plants were grown on the soil in well-watered control conditions. D, Plant survival rates and standard errors (SEs) (n = 3, where each replicate represents the survival plant rate of 30 plants/genotype). E, Soil moisture content was recorded during the water withholding (n = 5 positions/genotype/day). F, Relative water contents of *ahp4* and WT plants grown and subjected to water withholding treatment as described in (A-B). Data represent the means and SEs (n = 5/genotype). G, Electrolyte leakage rates of *ahp4* and WT plants grown and subjected to drought treatment as described in (A-B). Data represent the means and SEs (n = 5/genotype). Asterisks indicate significant differences between the two genotypes as determined by a Student's *t*-test (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001). DAS, days after stress.

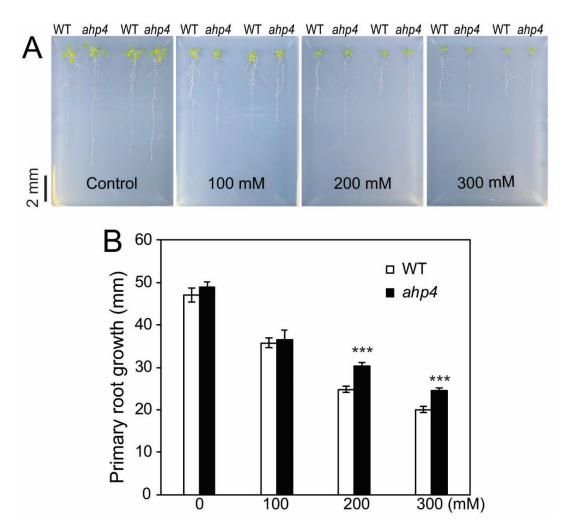
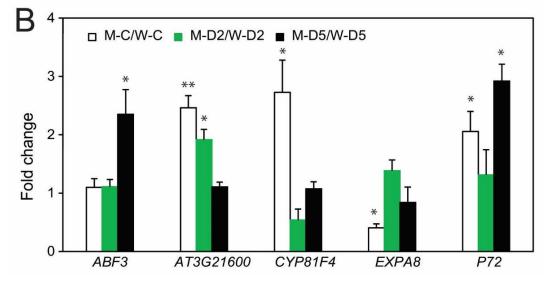
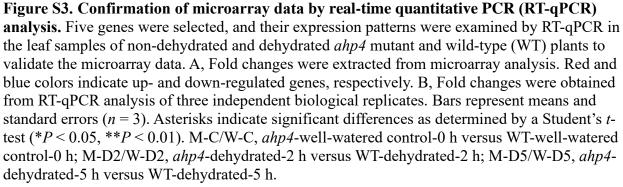


Figure S2. A representative assay of root growth of *ahp4* mutant and wild-type (WT) plants under normal and mannitol-induced osmotic stress conditions. A, Seven-day-old *ahp4* and WT plants grown on germination medium were transferred onto  $0.5 \times MS$  plates (1.2% w/v agar) with or without mannitol and grown for 7 additional days. B, Primary root growth of seven-dayold *ahp4* and WT plants grown under normal and mannitol conditions for 7 additional days. Data present the means and standard errors (n = 18 plants/genotype). Asterisks indicate significant differences between the two genotypes as determined by a Student's *t*-test (\*\*\*P < 0.001).

Δ	AGI	Gene description	M-C / W-C		M-D2 / W-D2		M-D5 / W-D5	
A			q-value	Fold change	q-value	Fold change	q-value	Fold change
	AT4G34000	ABF3	0.5779	0.6828	0.4793	3.4051	0.0202	5.1985
	AT3G21600	Dehydration-associated protein-like protein	0.0109	2.1202	0.0878	1.3197	0.1450	0.6364
	AT4G37410	CYP81F4	0.0285	9.8801	0.3303	0.5558	0.4122	0.6257
	AT2G40610	EXPA8	0.0385	0.4844	0.5403	1.2889	0.7679	0.7244
	AT5G66390	P72	0.0048	4.7093	0.6973	1.2666	0.3435	1.3136





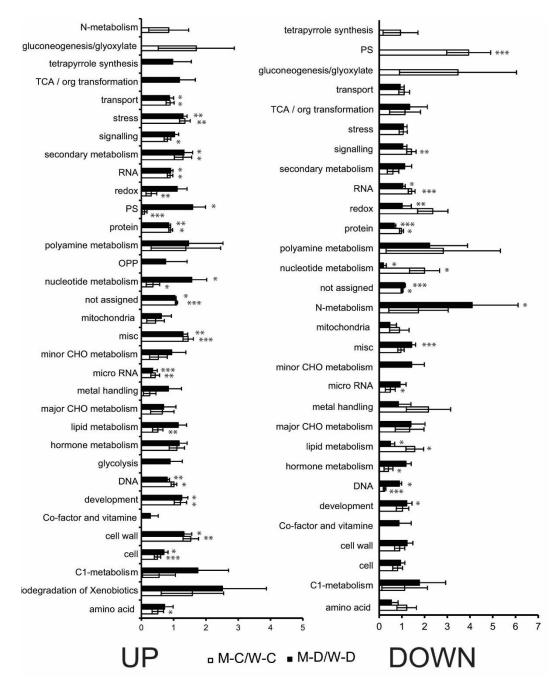
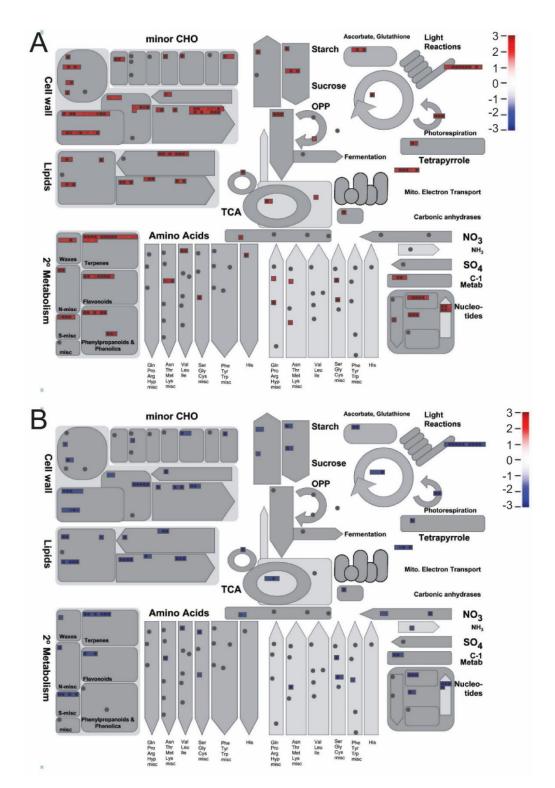


Figure S4. MapMan-based analysis of differentially expressed genes identified in *ahp4* mutant versus wild-type (WT) plants under normal (M-C/W-C comparison) and dehydration (M-D/W-D comparison) conditions. MapMan-based functional classification of the induced and repressed genes identified in M-C/W-C and M-D/W-D using the web-tool Classification Superviewer (bar.utoronto.ca) with normalized class score option (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001). CHO, carbohydrate; TCA, tricarboxylic acid; M-C/W-C comparison represents *ahp4* well-watered control versus WT well-watered control; M-D/W-D comparison represents M-D2/W-D2 and/or M-D5/W-D5; W-D2, WT dehydrated 2 h; W-D5, WT dehydrated 5 h; M-C, *ahp4* well-watered control; M-D2, *ahp4* dehydrated 2 h; M-D5, *ahp4* dehydrated 5 h.



**Figure S5. Metabolism-related overview of differentially expressed genes derived from** *ahp4* **versus wild-type under normal and dehydration conditions using MapMan.** Metabolism-related overview of up-regulated (A) and down-regulated (B) genes. Color bar in each panel indicates fold changes in gene expression.

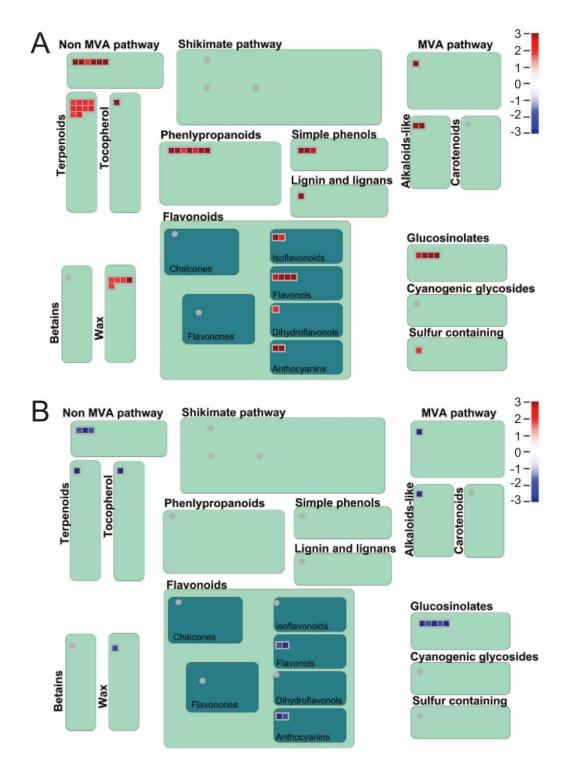


Figure S6. Secondary metabolism-related overview of differentially expressed genes identified in *ahp4* versus wild-type under normal and dehydration conditions using MapMan. A, Up-regulated and B, down-regulated genes. Red and blue colors indicate up- and down-regulated genes, respectively. Color bar in each panel indicates fold changes in gene expression.

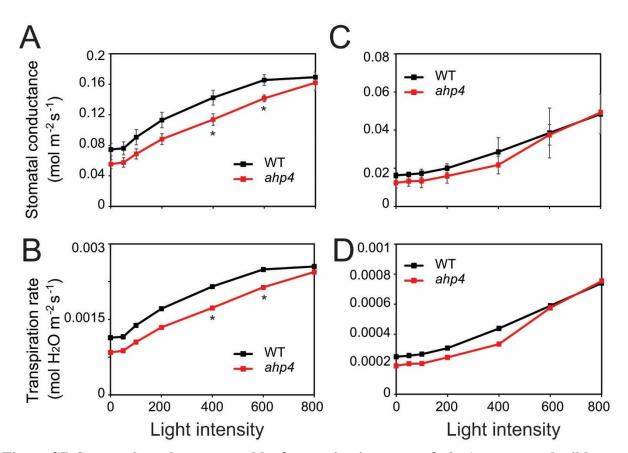


Figure S7. Stomatal conductance and leaf transpiration rates of *ahp4* mutant and wild-type (WT) plants under well-watered and dehydration conditions. A, Stomatal conductance, and B, leaf transpiration rates of 32-day-old *ahp4* and WT plants grown on soil under well-watered conditions. C, Stomatal conductance, and D, leaf transpiration rates of 32-day-old *ahp4* and WT plants grown on soil exposed to 30 minutes of dehydration treatment. Data represent the means and standard errors (n = 3). Asterisks indicate significant differences between the two genotypes as determined by a Student's *t*-test (\*P < 0.05).

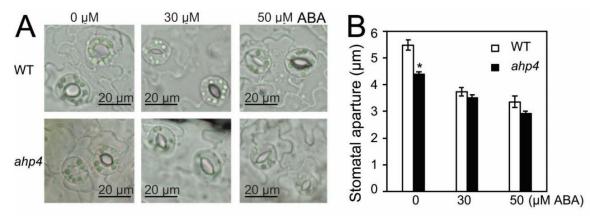


Figure S8. Comparison of stomatal apertures of *ahp4* mutant and wild-type (WT) plants under abscisic acid (ABA) treatment. A, Guard cells of 21-day-old *ahp4* and WT plants were treated with 0 (control), 30 and 50  $\mu$ M ABA for 1 h under light conditions. B, Average size of the stomatal aperture of rosette leaves from 21-day-old *ahp4* and WT plants in the presence or absence of ABA. Data represent the means and standard errors (n = 3 plants/genotype; for each plant the average of eight stomatal measurements from a single leaf was calculated). Asterisks indicate significant differences between the two genotypes as determined by a Student's *t*-test (\*P < 0.05).

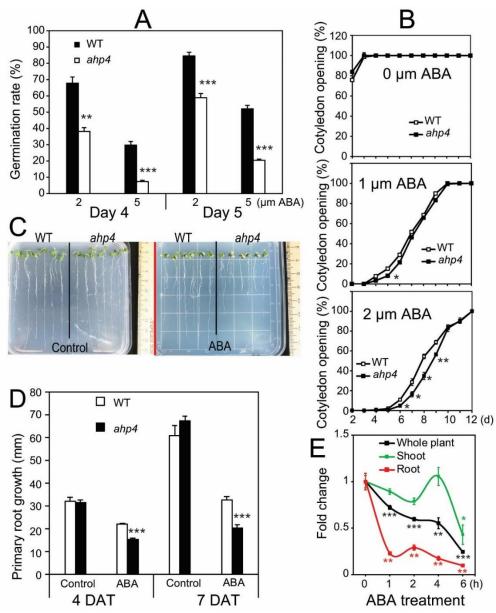


Figure S9. Germination assay of *ahp4* mutant and wild-type (WT) plants on medium supplemented with different concentrations of abscisic acid (ABA), and *AHP4* expression in WT plants treated with ABA. A, Germination rate of *ahp4* mutant and WT plants under exogenous ABA treatment. Data represent the mean and standard errors (SEs) (n = 8, where each replicate represents the radical emergence rate of  $\geq 18$  seeds/genotype). B, Cotyledon opening percentage of *ahp4* mutant and WT under exogenous ABA treatment. Data represent the mean and SEs (n = 3, where each replicate represents the cotyledon opening rate of 50 seeds/genotype). C, Seven-day-old *ahp4* and WT plants grown on germination medium were transferred onto  $0.5 \times MS$  plates (1.2% w/v agar) with or without 20 µM ABA for 7 days. D, Primary root growth of *ahp4* and WT plants under normal and ABA conditions as described in (C). Data present the mean and SEs (n = 18 plants/genotype). E, Expression of *AHP4* in 21-dayold WT plants treated with 50 µM ABA for indicated time period. Data represent the mean and SEs (n = 3). Asterisks indicate significant differences as determined by Student's *t*-test (\*P <0.05, \*\*P < 0.01, \*\*\*P < 0.001). DAT, days after transfer.

**Table S1.** Results of the comparative microarray analysis of leaves of *ahp4* mutant and wild-type plants under well-watered and dehydration conditions.

**Table S2**. List of up- and down-regulated genes in M-C/W-C comparison (|fold-change|  $\geq 2$ ; q-value < 0.05). A, List of up-regulated genes (fold change  $\geq 2$ , q-value <0.05) in M-C/W-C comparison. B, List of down-regulated genes (fold change  $\leq -2$ , q-value <0.05) in M-C/W-C comparison. M-C/W-C, *ahp4* well-watered control 0 h versus WT well-watered control 0 h.

**Table S3**. List of up- and down-regulated genes in various comparisons (|fold-change|  $\geq 2$ ; q-value < 0.05). A, List of up-regulated genes (fold change  $\geq 2$ , q-value < 0.05) in M-D/W-D comparison. B, List of up-regulated genes (fold change  $\geq 2$ , q-value < 0.05) in M-D2/W-D2 comparison. C, List of up-regulated genes (fold change  $\geq 2$ , q-value < 0.05) in M-D5/W-D5 comparison. D, List of down-regulated genes (fold change  $\leq -2$ , q-value < 0.05) in M-D/W-D comparison. E, List of down-regulated genes (fold change  $\leq -2$ , q-value < 0.05) in M-D2/W-D2 comparison. F, List of down-regulated genes (fold change  $\leq -2$ , q-value < 0.05) in M-D2/W-D2 comparison. F, List of down-regulated genes (fold change  $\leq -2$ , q-value < 0.05) in M-D5/W-D5 comparison. G, List of up-regulated genes (fold change  $\leq -2$ , q-value < 0.05) in M-D5/W-D5 comparison. G, List of up-regulated genes (fold change  $\geq -2$ , q-value < 0.05) in W-D/W-C comparison. H, List of down-regulated genes (fold change  $\geq -2$ , q-value < 0.05) in W-D/W-C comparison. M-D/W-D, *ahp4* dehydrated 2 and/or 5 h versus WT dehydrated 2 and/or 5 h; M-D2/W-D2, *ahp4* dehydrated 2 h versus WT dehydrated 2 h and/or 5 h versus *ahp4* well-watered control 0 h; W-D/W-C, WT dehydrated 2 h and/or 5 h versus WT well-watered control 0 h..

**Table S4**. Venn analysis of differentially expressed gene sets derived from various comparisons. A, List of up-regulated genes (fold change  $\geq 2$ , q-value <0.05) in M-C/W-C and W-D/W-C. B, List of up-regulated genes (fold change  $\geq 2$ , q-value <0.05) in M-C/W-C and W-D/W-C. C, List of down-regulated genes (fold change  $\leq -2$ , q-value <0.05) in M-C/W-C and W-D/W-C. D, List of down-regulated genes (fold change  $\leq -2$ , q-value <0.05) in M-D/W-D and W-D/W-C. D, List of down-regulated genes (fold change  $\leq -2$ , q-value <0.05) in M-D/W-D and W-D/W-C. M-C/W-C, *ahp4* well-watered control 0 h versus WT well-watered control 0 h; W-D/W-C, WT dehydrated 2 h and/or 5h versus WT well-watered control 0 h; M-D/W-D, *ahp4* dehydrated 2 and/or 5 h.

**Table S5**. List of photosynthesis-related genes in *ahp4* and wild-type (WT) leaves under normal and dehydration conditions.

**Table S6.** Primers used in RT-qPCR.