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2 **Supplemental Figure S1.** Cl⁻ content in xylem sap of wild-type-2 (wt-2) and *almt9-2* mutant
 3 plants upon 24 h salt treatment. Xylem sap Cl⁻ content was determined in hydroponically
 4 grown plants prior to (0 mM) and after NaCl treatment (100 mM). For each biological
 5 replicate (n = 4) xylem sap was collected and pooled from at least ten plants per genotype and
 6 treatment. Data are presented as means ± SEM. No statistically significant differences were
 7 detected between both genotypes by two-tailed Student's t-test (P < 0.05).

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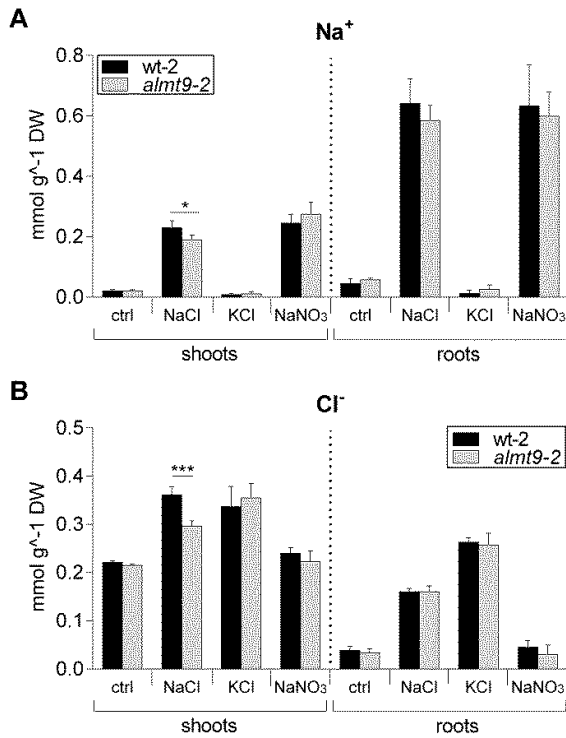
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22 **Supplemental Figure S2.** Na^+ and Cl^- contents in wild-type and *almt9* mutant plants upon
 23 treatment with different salt compositions. Na^+ (A) and Cl^- (B) content analysis in shoots
 24 ($n = 6$) and roots ($n = 5$) of hydroponically grown *almt9-2* mutants and the corresponding
 25 wild-type (wt-2) in response to 24 h treatment with control solution (ctrl), 100 mM NaCl,
 26 100 mM KCl and 100 mM NaNO₃. Data are means \pm SD. Asterisks indicate statistically
 27 significant differences from wt-2 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; two-tailed Student's
 28 t-test). DW, dry weight.

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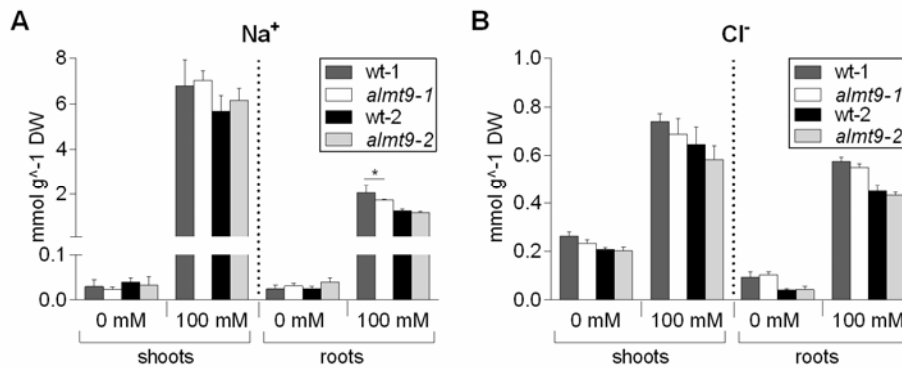
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40 **Supplemental Figure S3.** Na⁺ and Cl⁻ contents in wild-type and *almt9* mutant plants upon
 41 prolonged exposure to salinity. Na⁺ (A) and Cl⁻ (B) content analysis in shoots and roots of
 42 hydroponically grown *almt9-1* and *almt9-2* knock-out mutants and the corresponding
 43 wild-types (wt-1 and wt-2) upon seven days of treatment with control (0 mM) or NaCl
 44 (100 mM) solutions. Data are means ± SD of n ≥ 4 biological replicates. One-way ANOVA of
 45 each tissue and treatment and a pair-wise comparison was used for statistical analysis.
 46 Asterisks indicate significant differences from the corresponding wt (*P < 0.05, **P < 0.01,
 47 ***P < 0.001). DW, dry weight.

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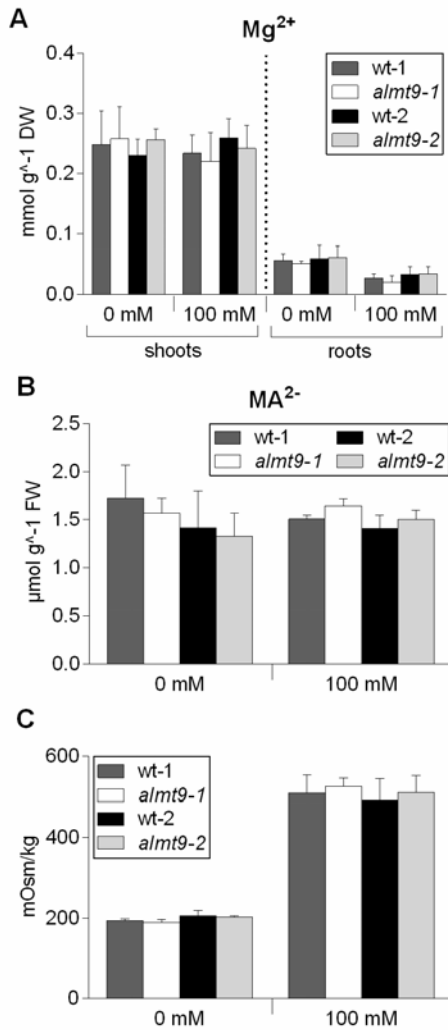
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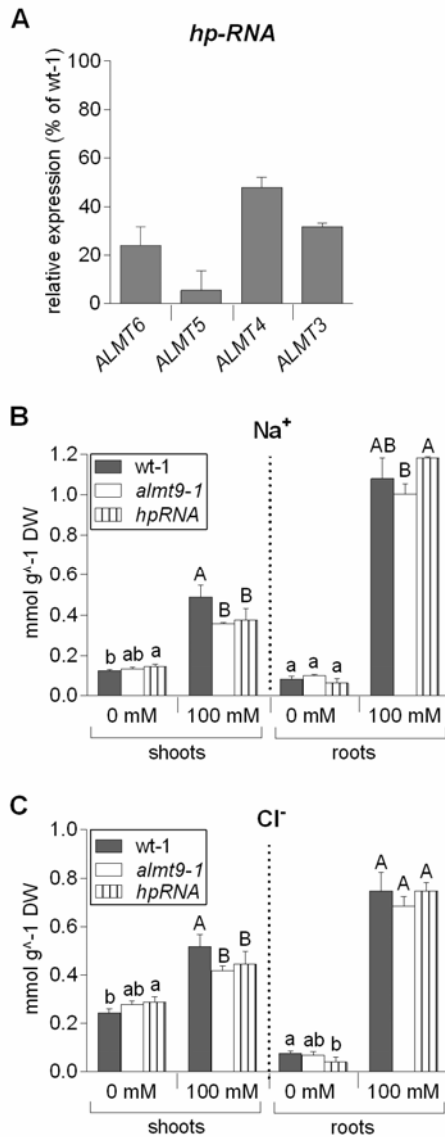
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66 **Supplemental Figure S4.** Mg^{2+} , MA^{2-} and osmotic contents in wild-type and *almt9* mutants
 67 during 24 h salt stress. Mg^{2+} (A; $n \geq 5$), MA^{2-} (B; $n \geq 4$), and osmotic (C; $n \geq 4$) contents
 68 were determined in shoots (and roots [A]) of hydroponically grown *almt9-1* and *almt9-2*
 69 mutants and the corresponding wild-types (wt-1 and wt-2) upon 24 h treatment with control (0
 70 mM) or NaCl (100 mM) solutions. Data are means \pm SD. No statistically significant
 71 differences were detected using one-way ANOVA of each tissue and treatment and a pair-
 72 wise comparison ($P < 0.05$). DW, dry weight; FW, fresh weight.

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77 **Supplemental Figure S5.** Evaluation of the transgenic hairpin-RNA-expressing line that
 78 targets clade II ALMTs for multiple transcriptional down-regulation (*hpRNA*). A, qRT-PCR
 79 analysis of the ALMT clade II members *ALMT6*, *ALMT5*, *ALMT4* and *ALMT3*. Transcript
 80 abundance was compared between wild-type-1 (wt-1) and *hpRNA* (*almt9-1* knock-out
 81 background). The data was normalized to expression levels of the target genes in wt-1. *ACT2*
 82 served as a reference gene. The SD was calculated from two biological replicates. B and C,
 83 Ion content analysis of Na^+ (B) and Cl^- (C) in shoots and roots of hydroponically grown wt-1,
 84 *almt9-1* and *hpRNA* upon 24 h treatment with control (0 mM) or NaCl (100 mM) solutions.
 85 Data are means \pm SD of n = 4 biological replicates. One-way ANOVA of each tissue and
 86 treatment and a Tukey-Kramer multiple comparison post-test was used for statistical analysis.

87 Different lowercase letters indicate significant differences in ion content ($P < 0.05$) under
88 control conditions, capital letters under salinity. DW, dry weight.

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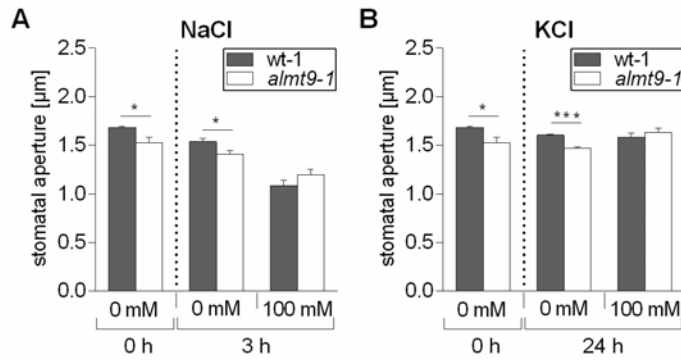
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108 **Supplemental Figure S6.** Stomatal movement in wild-type and *almt9* plants during NaCl and
 109 KCl stress. *In situ* assay of native stomatal apertures (see Material and Methods) using
 110 hydroponically grown wild-type-1 (wt-1) and *almt9-1* plants. Roots were exposed to control
 111 (0 mM) or stress solutions, and the stomatal aperture was measured before (0 h) and after
 112 treatment. A, Stomatal movement in response to 100 mM NaCl (100 mM) for 3 h. The
 113 measurement was conducted in parallel with the 24 h salinity treatment. Hence, the apertures
 114 before treatment are also shown in Fig. 5A. B, Stomatal movement in response to 100 mM
 115 KCl (100 mM) for 24 h. The measurement was conducted in parallel with the 24 h salinity
 116 treatment. Data of control conditions are also shown in Fig. 5A. The SEM was calculated
 117 from averages of $n \geq 4$ biological replicates. Asterisks indicate statistically significant
 118 differences from wt-1 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; two-tailed Student's t-test).

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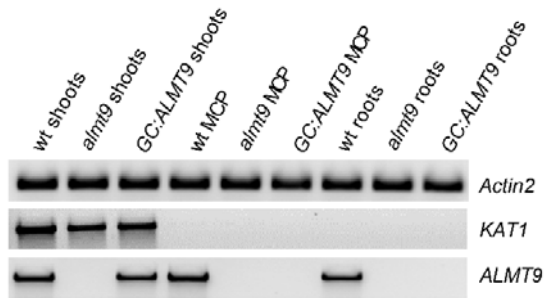
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130 **Supplemental Figure S7.** Evaluation of the guard cell-specific complementation line
 131 (*GC:ALMT9*) of *almt9-2* by PCR. For PCR amplification of *ACT2*, the guard cell-specific
 132 *KAT1* and *ALMT9* cDNA from wild-type-2 (wt), *almt9-2* and *GC:ALMT9* was used. cDNA
 133 was synthesized from mRNA extracted from either shoots, mesophyll cell protoplasts (MCP),
 134 or roots.

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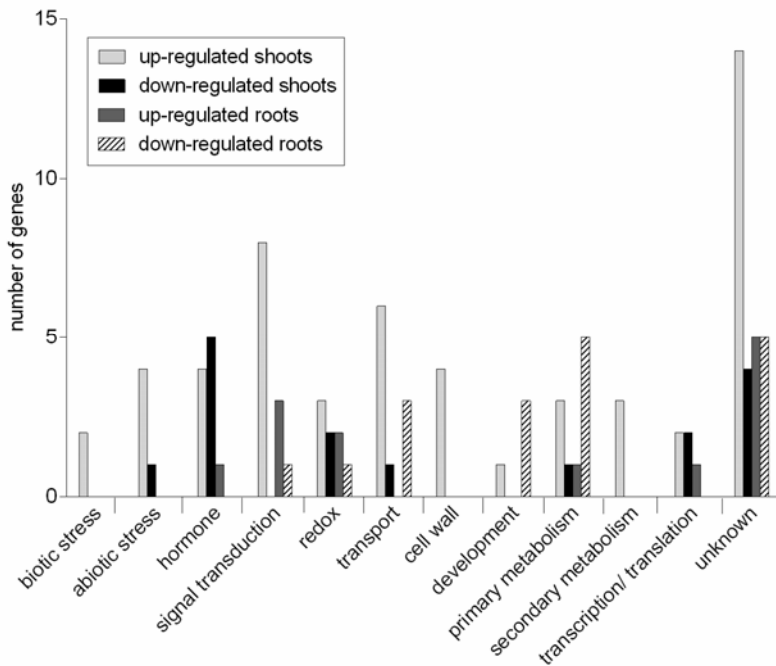
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143 **Supplemental Figure S8.** Biological functions of genes that are differentially expressed in
 144 *almt9-1* exclusively under salinity. Genes that show a differential expression exclusively
 145 under salinity conditions (24 h, 100 mM NaCl) in *almt9-1* shoots and roots (for selection
 146 requirements see Material and Methods section) were arranged in selected functional
 147 categories. Up-regulated, genes with higher expression in *almt9-1* compared to wild-type-1;
 148 down-regulated, genes with lower expression in *almt9-1*.

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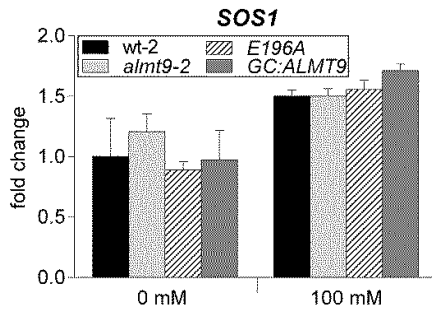
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163 **Supplemental Figure S9.** Expression analysis of *SOS1* upon 24 h salt treatment. Expression
 164 of *SOS1* was determined in roots of wild-type-2 (wt-2), *almt9-2*, and the complemented lines
 165 *E196A* and *GC:ALMT9* upon exposure to control (0 mM) or NaCl (100 mM) conditions for
 166 24 h by qRT-PCR. Each data point was derived from n = 3 biological replicates and is shown
 167 as mean ± SD. The data were normalized to the expression level of *SOS1* in wt-2 under
 168 control conditions. *ACT2* served as a reference gene. No statistically significant differences
 169 were detected using one-way ANOVA and Tukey-Kramer multiple comparison post-test for
 170 each treatment ($P < 0.05$).

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Supplemental Table S1. Ion contents of wild-type and *almt9* mutant plants upon 24 h salinity.

Ion	NaCl [mM]	Tissue	Mean \pm SD ^a			
			wild-type-1	<i>almt9-1</i>	wild-type-2	<i>almt9-2</i>
Na ⁺	0	Shoots	0.023 \pm 0.005	0.018 \pm 0.005	0.062 \pm 0.009	0.059 \pm 0.005
	100	Shoots	0.455 \pm 0.053	0.366 \pm 0.028**	0.355 \pm 0.012	0.273 \pm 0.035*
	0	Roots	0.033 \pm 0.009	0.041 \pm 0.012	0.045 \pm 0.014	0.048 \pm 0.015
	100	Roots	0.853 \pm 0.098	0.804 \pm 0.168	0.845 \pm 0.150	0.763 \pm 0.178
Cl ⁻	0	Shoots	0.221 \pm 0.032	0.263 \pm 0.027*	0.204 \pm 0.122	0.209 \pm 0.013
	100	Shoots	0.547 \pm 0.064	0.406 \pm 0.023***	0.534 \pm 0.035	0.451 \pm 0.017*
	0	Roots	0.077 \pm 0.026	0.075 \pm 0.028	0.082 \pm 0.011	0.085 \pm 0.034
	100	Roots	0.749 \pm 0.067	0.737 \pm 0.079	0.763 \pm 0.143	0.733 \pm 0.083
K ⁺	0	Shoots	1.295 \pm 0.123	1.393 \pm 0.083	1.207 \pm 0.052	1.287 \pm 0.084
	100	Shoots	1.200 \pm 0.058	1.186 \pm 0.069	1.238 \pm 0.038	1.256 \pm 0.081
	0	Roots	0.947 \pm 0.049	0.996 \pm 0.085	1.052 \pm 0.217	1.029 \pm 0.310
	100	Roots	0.668 \pm 0.188	0.648 \pm 0.124	0.740 \pm 0.132	0.569 \pm 0.099
NO ₃ ⁻	0	Shoots	0.163 \pm 0.007	0.157 \pm 0.012	0.168 \pm 0.021	0.166 \pm 0.028
	100	Shoots	0.160 \pm 0.013	0.156 \pm 0.016	0.162 \pm 0.015	0.170 \pm 0.011
	0	Roots	0.060 \pm 0.004	0.060 \pm 0.002	0.054 \pm 0.003	0.055 \pm 0.001
	100	Roots	0.055 \pm 0.006	0.055 \pm 0.006	0.057 \pm 0.004	0.055 \pm 0.002

^a Concentrations are shown in [mmol/ g dry weight] for Na⁺, Cl⁻, and K⁺. Concentrations are shown in [mmol/ g fresh weight] for NO₃⁻. The results are illustrated in Fig. 2. Data of n \geq 5 biological replicates derived from two independent experiments are shown. One-way ANOVA of each tissue and treatment and a pair-wise comparison was used for statistical analysis. Asterisks indicate significant differences from the corresponding wild-type (*P < 0.05, **P < 0.01, ***P < 0.001).

Supplemental Table S2. List of differentially expressed genes between wild-type-1 and

almt9-1 in shoots exclusively under salinity.

Gene ID	Description/ Name	Log ₂ Ratio	p-value
AT2G18190	P-loop containing nucleoside triphosphate hydrolases superfamily protein	4.674	1.58E-05
AT3G26830	Cytochrome P450 enzyme (CYP71B15/ PAD3)	4.411	1.32E-05
AT2G04040	MATE-related detoxification efflux carrier (DTX1)	3.998	4.70E-05
AT1G29715	Pseudogene	3.98	5.93E-05
AT1G66390	MYB DOMAIN PROTEIN 90 (MYB90/ PAP2)	3.74	0.003046
AT1G26380	FAD-binding Berberine family protein	3.478	7.06E-05
AT4G27140	Seed storage albumin (SESA1)	-3.441	0.001111
AT3G28580	P-loop containing nucleoside triphosphate hydrolases superfamily protein	3.371	0.0002297
AT1G05680	Uridine diphosphate glycosyltransferase (UGT74E2)	3.338	2.90E-07
AT1G13520	Protein of unknown function (DUF1262)	3.313	0.0007433
AT3G44300	Nitrilase (NIT2)	3.217	3.46E-05
AT2G04070	Putative MATE-related efflux carrier	3.05	0.001947
AT1G62440	LEUCINE-RICH REPEAT/EXTENSIN 2 (LRX2)	2.792	7.26E-07
AT5G19100	Eukaryotic aspartyl protease family protein	-2.774	0.002622
AT5G01550	Lectin receptor kinase subfamily A4 (LecRKA4.2)	2.724	0.001441
AT4G04540	Cysteine-rich receptor like protein kinase (CRK39)	2.675	0.002616
AT2G30750	Putative cytochrome P450 (CYP71A12)	2.619	0.008339
AT1G17960	Threonyl-tRNA synthetase	2.573	0.0003076
AT2G04050	Putative MATE-related efflux carrier	2.535	3.38E-05
AT1G16420	Metacaspase (MCP2E/ MC8)	2.497	0.001789
AT1G63030	ERF/AP2 transcription factor family (DDF2)	-2.258	0.0005246
AT2G38240	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	-2.247	2.76E-05
AT2G12930	Gypsy-like retrotransposon family	-2.199	0.003696
AT3G28740	Cytochrome P450 family (CYP81D11)	2.19	0.009735
AT1G21240	Wall-associated kinase (WAK3)	2.093	2.62E-07
AT1G49310	Unknown protein	2.079	0.00275

AT4G20000	VQ motif-containing protein	2.045	0.006998
AT5G10970	C2H2 and C2HC zinc fingers superfamily protein	-2.031	0.0006944
AT2G18193	P-loop containing nucleoside triphosphate hydrolases superfamily protein	2.007	0.0001036
AT5G25260	SPFH/Band 7/PHB domain-containing membrane- associated protein family	1.957	0.001054
AT1G17170	Glutathione transferase (GSTU24)	1.956	0.0002155
AT3G63380	AUTO-INHIBITED CA²⁺ ATPASE 12 (ACA12)	1.865	0.004314
AT2G31910	Putative Na⁺/H⁺ antiporter family (CHX21)	-1.848	0.004987
AT3G13950	Unknown protein	1.803	0.002431
AT3G29250	NAD(P)-binding Rossmann-fold superfamily protein (SDR4)	1.764	0.002492
AT1G12610	ERF/AP2 transcription factor family (DDF1)	-1.717	1.28E-08
AT3G09640	Cytosolic ascorbate peroxidase (APX2/ APX1B)	-1.669	0.0004518
AT5G51440	HSP20-like chaperones superfamily protein	1.664	0.007465
AT2G47000	Subfamily B ABC-type auxin efflux transporter (ABCB4/ MDR4/ PGP4)	1.636	0.001916
AT1G43910	P-loop containing nucleoside triphosphate hydrolases superfamily protein	1.597	0.005181
AT3G25010	Receptor like protein (RLP41)	1.593	1.29E-06
AT4G04490	Cysteine-rich receptor like protein kinase (CRK36)	1.583	0.001103
AT1G61810	Beta-glucosidase (BGLU45)	1.58	0.003976
AT2G29350	Senescence-associated gene (SAG13)	1.564	1.40E-07
AT3G57240	Glycosyl hydrolase family 17 (BG3)	1.514	5.12E-05
AT4G12410	SAUR-like auxin-responsive protein family (SAUR35)	-1.49	0.002346
AT4G25000	ALPHA-AMYLASE-LIKE (AMY1)	1.482	3.40E-05
AT5G60250	Zinc finger (C3HC4-type RING finger) family protein	1.473	0.0005328
AT1G57630	Toll-Interleukin-Resistance (TIR) domain family protein	1.464	0.00685
AT4G34410	ERF/AP2 transcription factor family (ERF109)	-1.458	2.87E-12
AT2G29460	Glutathione transferase (GSTU4)	1.415	0.007186

AT3G10320	Glycosyltransferase family 61 protein (MUCI21)	1.397	0.006958
AT5G52020	ERF/AP2 transcription factor family	-1.396	8.35E-06
AT1G53540	HSP20-like chaperones superfamily protein	-1.386	0.002712
AT1G76930	Extensin (EXT1/EXT4)	1.38	0.0007415
AT4G00700	C2 calcium/lipid-binding plant phosphoribosyltransferase family protein	1.378	0.004223
AT4G24000	Cellulose synthase (CSLG2)	1.324	0.002603
AT5G02490	Heat shock protein 70 (Hsp 70) family protein (HSP70-2)	1.285	9.44E-05
AT4G23140	Cysteine-rich receptor like protein kinase (CRK6)	1.229	8.88E-17
AT4G37370	Cytochrome P450 family (CYP81D8)	1.198	0.006318
AT5G18270	NAC domain containing protein (ANAC087)	1.184	0.002222
AT3G57765	Small nuclear RNA (U2.3)	-1.149	0.00242
AT1G14780	MAC/Perforin domain-containing protein	1.125	8.42E-05
AT1G15405	Unknown gene	-1.12	0.009702
AT4G27654	Unknown protein	-1.117	2.97E-08
AT3G28890	Receptor like protein (RLP43)	1.085	0.0001529
AT2G29110	Ligand-gated ion channel subunit family (GLR2.8)	1.065	0.007163
AT2G03760	Brassinosteroid sulphotransferase (SOT12/ ST1)	1.031	0.0008528
AT1G58225	Unknown protein	1.015	0.0008908
AT1G35230	Arabinogalactan protein (AGP5)	1.001	0.0007468

List of genes that are exclusively significantly up- or down-regulated under salinity conditions (24 h, 100 mM NaCl) in *almt9-1* shoots (for selection requirements see Material and Methods section). Positive \log_2 ratios correspond to transcriptional up-regulation in *almt9-1*, negative \log_2 ratios to transcriptional down-regulation. Names and descriptions are according to TAIR. Genes mentioned in the study are highlighted.

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Supplemental Table S3. List of differentially expressed genes between wild-type-1 and

almt9-1 in roots exclusively under salinity.

Gene ID	Description/ Name	Log ₂ Ratio	p-value
AT4G04223	Unknown gene	-3.337	6.03E-22
AT5G24140	Squalene monooxygenase (SQP2)	-2.981	0.0003986
AT5G02330	Cysteine/Histidine-rich C1 domain family protein	-2.502	0.0003037
AT3G01015	TPX2 (targeting protein for Xklp2) protein family	2.228	0.001345
AT1G19415	Copia-like retrotransposon family	-2.145	0.009452
AT2G30766	Unknown protein	2.002	3.22E-06
AT1G51030	Unknown protein	-1.998	0.001067
AT4G38590	Putative beta-galactosidase (BGAL14)	-1.95	0.005079
AT4G15690	Thioredoxin superfamily protein	1.944	0.0003804
AT5G17040	UDP-Glycosyltransferase superfamily protein	-1.794	0.0023
AT1G71890	Sucrose transporter (SUC5)	-1.778	0.005836
AT4G23320	Cysteine-rich receptor like protein kinase (CRK24)	1.777	0.003051
AT5G03310	SAUR-like auxin-responsive protein family (SAUR44)	1.624	0.003661
AT5G11410	Protein kinase superfamily protein	1.545	0.006612
AT4G23210	Cysteine-rich receptor like protein kinase (CRK13)	-1.531	0.0003196
AT1G14345	NAD(P)-linked oxidoreductase superfamily protein	-1.5	0.003167
AT5G27220	Frigida-like protein	1.484	0.009174
AT1G73220	Organic cation/carnitine transporter (OCT1)	-1.483	0.0001871
AT3G53040	Putative late embryogenesis abundant (LEA) protein	-1.477	0.003184
AT2G48121	Unknown protein	1.459	0.009511
AT1G33320	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein	-1.441	0.0002151
AT2G03710	SEPALLATA 4 (SEP4/ AGL3)	-1.224	0.0006572
AT1G02250	NAC family of transcription factors (ANAC005)	1.15	0.003483
AT3G22560	Acyl-CoA N-acyltransferases (NAT) superfamily protein	-1.146	0.00112
AT3G60090	VQ motif-containing protein	-1.129	0.001143
AT2G39510	Nodulin MtN21-like transporter family protein (UMAMIT14)	-1.112	0.003848

AT2G02061	Nucleotide-diphospho-sugar transferase family protein	1.069	0.00585
AT4G15360	Cytochrome P450 family (CYP705A3)	1.031	0.0006057
AT1G25150	F-box family protein	1.028	0.002636
AT4G17660	Protein kinase superfamily protein	1.019	0.00087
AT5G44565	Unknown protein	-1.019	0.001466

List of genes that are exclusively significantly up- or down-regulated under salinity conditions (24 h, 100 mM NaCl) in *almt9-1* roots (for selection requirements see Material and Methods section). Positive \log_2 ratios correspond to transcriptional up-regulation in *almt9-1*, negative \log_2 ratios to transcriptional down-regulation. Names and descriptions are according to TAIR. Genes mentioned in the study are highlighted.

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Supplemental Table S4. Primers used in that study.

Primer Description	5' - 3' Sequence
qPCR-ACT2-for	TGGAATCCACGAGACAACCTA
qPCR-ACT2-rev	TTCTGTGAACGATTCCTGGAC
qPCR-SOS1-for	CTCTTCGTCGGAATGTCTCTG
qPCR-SOS1-rev	ACGAATTCCATGGCCGATCTTT
qPCR-ALMT9-for	ACCTAATCCGGATCTTAGTCGATACT
qPCR-ALMT9-rev	TCACCGAATAAAGTGGAAAGCTCAG
qPCR-ALMT6-for	CCGTTGCATGATGCTAGTAAATAC
qPCR-ALMT6-rev	TGATGATGGTTTGCTCGAAA
qPCR-ALMT5-for	GAGCCGCTTCAAGATGCTAGTA
qPCR-ALMT5-rev	ATGACTTCTTCAAACCTCTCCTGCT
qPCR-ALMT4-for	TGACGCTAGCAAGTATGCTGTT
qPCR-ALMT4-rev	CTTCAAATTCTCCAGCTGAAACAGA
qPCR-ALMT3-for	GGCTTATCCTACAGAGCAGAGGCT
qPCR-ALMT3-rev	TCAGAGCCAAACCCATCTTC
qPCR-HKT1;1-for	TGGTTGGATCGTTGTTTCAA
qPCR-HKT1;1-rev	CGGAATCATCATCTCCTCCT
qPCR-CHX21-for	ACGCAACTGTCTGTCTGCTAA
qPCR-CHX21-rev	CGAGAGCTAAGTTTGCGAATG
qPCR-DTX1-for	CCAATACGGAATCCCATCAG
qPCR-DTX1-rev	ATTCCCAGCTCCCAAATTGT
hpRNA-for	GATCGGATCCCTCGAGGAATACGAGAGAATACCG TCG
hpRNA-rev	GATCATCGATGGTACCCAGCTTTCTGAGTTGACA AGAAG
ALMT9 _{pro} :ALMT9 _{E196A} -for	TAGTTGGAATGGGTTTCGAAAAATTACAATTGTTT CCTCT
ALMT9 _{pro} :ALMT9 _{E196A} -rev	TTATGGAGTTGGGTTTCGAACCATCCCAAACACC TACGA
SDM _{E196A} -for	GATGAAAGCTTATGCATACGGGTTCCGGG
SDM _{E196A} -rev	CCCGGAACCCGTATGCATAAGCTTTCATC
ALMT9 _{pro} -for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAA ATTACAATTGTTTCCTCT
ALMT9 _{pro} -rev	GGGGACCACTTTGTACAAGAAAGCTGGGTGTACT

	AGACGGATTCTCAAAG
ALMT9 _{GC} -for	GGCGCGCCATGGCGGCGAAGCAAGGTTC
ALMT9 _{GC} -rev	GACTAGTCTTACATCCCAAAACACCTAC
KAT1-for	ATGTCGATCTCTTGGACTCG
KAT1-rev	ATTTGATGAAAAATACAAATGATCACC

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