

Supporting Information (SI Appendix)

AtPep3 is a hormone-like peptide that plays a role in the salinity stress tolerance of plants

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Supporting Text

Plant material and growth conditions

Arabidopsis thaliana ecotype Columbia-0 was used in the majority of the experiments. Single and double mutants of *pepr* plants were also used from a previously described study (1). Plants were grown on agar plates containing Murashige and Skoog (MS) medium with a final concentration of 1 % sucrose at 22 °C under long day (16h/8h light/dark) conditions at a light intensity of 50–75 $\mu\text{molm}^{-2}\text{s}^{-1}$. Ten- to fourteen-day-old *Arabidopsis* plants were used in the salinity stress assays.

Generation of AtPROPEP3/AT13-overexpressing and RNAi plant lines

In order to generate transgenic plants overexpressing the various small coding genes, abiotic-stress-related small coding genes were introduced into pMDC32, a vector that possesses a double 35S promoter (2). For the functional studies, knock-down RNAi lines of the AtPROPEP3/AT13 gene was constructed as follows. The RNAi construct was generated using a method described in Higuchi et al (3). The appropriate RNAi fragment was prepared using 2 oligos: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTTTTATAGTCATTTGTTTCGAA TATGAATTGGATTAATAAAGATCGAATGGCAATGAGTAAAGGAGAA-3' and 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTTTTTTTATAGTCATTTGTTTCGAATATGA ATTGGATTAATAAAGATCGATTCTCCTTTACTCATTGCCAT-3' and the fragment was then introduced into pMDC32. The recombinant binary vector was subsequently introduced into *Agrobacterium tumefaciens* (strain GV3101) and inserted into the *Arabidopsis* genome using the floral-dip method (4). The level of AtPROPEP3/AT13 mRNA expression in all of the transgenic lines was confirmed by qRT-PCR.

Salinity stress assay

Salinity tolerant lines among the transgenic lines overexpressing the small coding genes were identified using a plate-based stress test and a root elongation assay. The initial screening utilizing

the plate-based test was conducted on T2 generation plants of at least 10 lines for each of the small coding genes. For the assessment of salt tolerance, one-week-old plants were transferred to MS plates containing 200 mM NaCl. The plants were grown at 22 °C for a period of 10 to 14 days after which the number of surviving plants was counted. P-values were calculated using the chi square test in order to identify lines with significant levels of salt tolerance. Small coding genes where more than two lines of ox plants exhibited a p-value of < 0.05 were considered for further screening.

Liquid culture-based salinity stress test

WT, AtPROPEP3/AT13-ox, or RNAi plants were grown in liquid MS medium for 10 days at 22 °C under long day conditions. The salinity stress test was performed by replacing the liquid medium with new medium containing 150 mM or 125 mM NaCl. The plants were grown at 22 °C for a period of 10 to 14 days and survivorship was subsequently evaluated by measuring the chlorophyll content in the plants.

Measurements of chlorophyll content

Chlorophyll content was measured using a method as previously described in Myouga et al. (5). Whole seedlings of salinity stressed plants growing in liquid culture were used for the measurements. Chlorophyll was extracted with dimethylformamide and the absorbance of the extracted solution was measured at A646.8 and A663.8 with a SmartSpec Plus (Bio-Rad, Hercules, CA, USA). Chlorophyll content was calculated using the following formula: (Chl a + b = 17.67A646.8 + 7.12A663.8).

Peptide design and treatment assay

Synthetic AT13 peptide fragments were purchased from Eurofins (Ebersberg, Germany) and the designs of the peptide fragments are summarized in Table S3. The synthetic peptides were dissolved in dimethyl sulfoxide (DMSO) and peptide treatments were conducted using the liquid culture-based salinity stress assay. WT or RNAi seedlings were grown in liquid MS medium for 7 days and the medium was then replaced with MS medium containing each peptide fragment and grown for an additional 3 days to serve as a peptide pre-treatment. The salinity stress test was performed by replacing the peptide-containing medium with MS medium containing NaCl and the peptide after the pre-treatment. The plants were grown at 22 °C for a period of 10 to 14 days and survivorship was evaluated by measuring the chlorophyll content in the plants.

Microarray analysis

Total RNA was extracted from two biological replicates of eleven-day-old AtPROPEP3/AT13-ox plants, plants treated with AtPEP3/AT13 peptide, and non-treated WT plants with Plant RNA Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 500 ng of total RNA was

used for cRNA labeling using a Quick Amp labeling kit (Agilent Technologies, Palo Alto, CA, USA) and the labeled cRNA was then hybridized to a 44K custom array (Agilent Technologies) as previously described (6). Arrays were scanned with a microarray scanner (G2505B, Agilent Technologies). The microarray data are available on the GEO website (GEO ID: GSE78515). The array intensities were processed using the Bioconductor (www.bioconductor.org) package in the R software environment (www.r-project.org). Hybridization intensities in the arrays were normalized among different arrays by quantile normalization. Normalized intensities of two replicates were compared with LIMMA in the R software environment (www.r-project.org). Up- and down-regulated genes were defined as genes with significantly higher and lower intensities, respectively, in AtPROPEP3/AT13-ox plants vs. non-treated WT plants or in AtPEP3/AT13 peptide treated plants vs. non-treated WT plants (False Discovery Rate <0.05). GO assignments for Arabidopsis genes were obtained from TAIR (www.arabidopsis.org) and used for determining statistically overrepresented GO Categories in either AtPROPEP3/AT13-ox plants or plants treated with AtPEP3/AT13 peptide. Only GO categories belonging to biological processes were analyzed among the top three GO categories. The number of regulated genes in either AtPROPEP3/AT13-ox plants or plants treated with AtPEP3/AT13 peptide was obtained in each of the GO categories. In each GO category, the expected values were then compared with the observed values using the χ^2 test to determine whether the ratio of observed numbers of regulated genes to those of non-regulated genes was significantly higher than the expected ratio. To correct for multiple testing, a moderated P value (q) was estimated from raw χ^2 test P-values using Q-VALUE software (7). The null hypothesis was rejected if q values were < 0.05.

Sample preparation for MS/MS analysis

Two-week-old wild-type seedlings were treated with or without application of 200mM NaCl. Total protein was extracted with Lysis Buffer (150 mM NaCl, 1% Triton X-100, 50 mM Tris-HCl (pH 8.0) containing cOmplete protease inhibitor cocktail (Roche, Basel, Switzerland)). Sample preparation was performed as previously described (8). The same amount of total protein samples was separated using SuperSep Ace 15 % (Wako, Osaka, Japan) precast gels and stained using SilverSNAP for a Mass Spectrometry kit (Thermo Fisher Scientific, Waltham, MA, USA). The lower area of gels (below approximately 20 kDa) was cut out and destained using the same SilverSNAP kit. Protein samples were partially digested by trypsin at 37 °C for 1 h after reduction with dithiothreitol and alkylation with iodoacetamide.

LC-MS/MS analysis and database searching

Purified peptide samples were analyzed using a HTC-PAL/Paradigm MS4 system coupled to a LTQ-Orbitrap XL (Thermo Fisher Scientific) mass spectrometer. Details of LC-MS/MS analysis and

database searching were previously described (8). An amino acid sequence of the AtPEP3/AT13 peptide (EIKARGKNKTKPTPSSGKGGKHN) was searched from obtained spectra using the MASCOT server (version 2.2). The mascot search parameters were as follows: set off the threshold at 0.05 in the ion-score cut-off, peptide tolerance at 10 ppm, MS/MS tolerance at ± 0.6 Da, peptide charge of 2+ or 3+, semiTrypsin as the enzyme allowing up to seven missed cleavages.

References

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2. Curtis MD & Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* 133(2):462-469.
3. Higuchi M, *et al.* (2009) Simple construction of plant RNAi vectors using long oligonucleotides. *Journal of plant research* 122(4):477-482.
4. Clough SJ & Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* 16(6):735-743.
5. Myouga F, Motohashi R, Kuromori T, Nagata N, & Shinozaki K (2006) An Arabidopsis chloroplast-targeted Hsp101 homologue, APG6, has an essential role in chloroplast development as well as heat-stress response. *Plant J* 48(2):249-260.
6. Hanada K, *et al.* (2013) Small open reading frames associated with morphogenesis are hidden in plant genomes. *Proc Natl Acad Sci U S A* 110(6):2395-2400.
7. Storey JD, Bass AJ, Dabney A, & Robinson D (2015) qvalue: Q-value estimation for false discovery rate control. R package version 2.2.2.
8. Fujiwara M, *et al.* (2014) Interactomics of Qa-SNARE in Arabidopsis thaliana. *Plant Cell Physiol* 55(4):781-789.

Supporting Figure Legends

Fig. S1. Homology search and expression analysis of small coding gene candidates. (A) Box plots of the numbers of homologs in other plant species of small coding genes analyzed in the present study (YES) and not analyzed in the present study (NO). (B) Box plots of fold change of small coding genes analyzed in the present study (YES) and not analyzed in the present study (NO). Gene expression levels and fold change were calculated from our previously published microarray data (6). (C) Summary of peptide candidates containing secreted signal for transgenic Arabidopsis plants.

Fig. S2. Salinity stress tolerance of overexpressing small coding gene. Image of salinity stress tolerance of *AT5*, *AT23*, *AT13* and *AT23-ox* plants (T2 generation) by plate-based salinity stress test (top) and survivability (bottom). One-week-old plants were transferred to 200 mM NaCl containing MS plates and grown for 10 to 14 days. Ox plants (red) were compared with WT and *T. halophila* (positive control). This test revealed that several small coding genes overexpressing plants exhibited salinity stress tolerance and summarized in Table S2. These are the candidates of peptide hormones or -like compounds.

Fig. S3. Primary root growth of overexpressing small coding gene under salinity stress. Image of root growth under salinity stress of *AT5*, *AT23*, *AT13* and *AT23-ox* plants (T3 generation) compared with WT (left) and the measurement of primary root (right). One-week-old plants were transferred to 150 mM NaCl containing MS plates and grown on vertical for 14 days. This test revealed that these 4 small coding genes are the candidates of peptide hormones or -like compounds for salinity stress tolerance.

Fig. S4. Fold change of small coding genes under salinity stress treatment. Gene expression levels and fold change were calculated from our previously published microarray data (6). Box plots indicated the 4 candidates of salinity stress tolerance related small coding genes, *AT5*, *AT12*, *AT13* and *AT23* (+) compared with the other 11 genes (-) used for screening in this study. Salinity stress tolerance related small coding genes showed higher induction under salinity stress treatment.

Fig. S5. Specific expression of AT13 mRNA. Diagrammatic representation of mRNA expression of *AT13* in different tissues, light and environmental stress conditions as determined by microarray analysis. The diagram was obtained from the website, HANADB-AT (<http://evolver.psc.riken.jp/seiken>). *AT13* mRNA was specifically induced by salinity stress.

Fig. S6. Expression level of *AtPROPEPs* under salinity stress treatment. (A) Fold changes of *AtPROPEP* genes were analyzed from our previously published microarray data (6). Two-week-old plants were treated with 200 mM NaCl for 2 (blue) and 6 hours (black). (B) Normalized intensities of *AtPROPEP* genes from microarray data are visualized. The y-axis represents quantile-normalized hybridization intensities from the microarray experiments.

Fig. S7. AT13 peptide induce salinity stress tolerance. Whole plant image of peptide treatment test (three replicate) and chlorophyll measurement after test. Seven-day-old plants were pre-treated with 10 μ M peptide for 3 days and then 150 mM NaCl was treated with peptides under liquid culture-based salinity stress test. Plants were grown for 10 days and chlorophyll contents were measured to evaluate the salinity stress tolerance. DMSO treatment was as a negative control.

Fig. S8. Expression levels of the *PEPCI* gene in response to Pep3 treatment and overexpression of *At13*. In relative comparison to treatment with DMSO (normalized value = 1), a significant up-regulation of *PEPCI* (10.10) was observed in response to the application of Pep3. When relative expression values were compared to wild-type plants (normalized value = 1), the overexpression of *At13* resulted in up-regulation of *PEPCI* (3.02), whereas RNAi suppressed its expression (0.51).

Fig. S9. AT13RNAi exhibited salt sensitive phenotype. Whole plant image of *AT13*RNAi plants under liquid culture-based salinity stress test. Ten-day-old plants were treated with 150 mM NaCl and grown on 10 more days. The sensitive phenotype of *AT13*RNAi plants were complemented with treatment of AT13 peptide. Chlorophyll contents were measured to evaluate tolerance and sensitivity.

Fig. S10. Treatment with the AT13 peptide does not affect osmotic stress tolerance. Representative image of plants under a liquid culture-based osmotic stress test. Seven-day-old wild-type plants were pre-treated with 100 nM peptide for 3 days. Subsequently, treatment with 500 mM mannitol was utilized for an osmotic stress with peptides under a liquid culture-based test. Plants were grown for 10 days and fresh weights were determined to evaluate the osmotic stress tolerance.

Fig. S11. Peptide design of AT13 fragments for treatment assay. Amino acid sequence of AT13-5 fragment and different length of synthetic peptide fragments used in the salt treatment assay.

Fig. S12. AT13 peptide induced salinity stress tolerance. Images of representative plants under

liquid culture-based salinity stress test. Seven-day-old plants were pre-treated with different length of 100 nM AT13 peptides for 3 days and then 150 mM NaCl was treated with peptides under liquid culture-based salinity stress test. Chlorophyll contents were measured to evaluate tolerance and sensitivity.

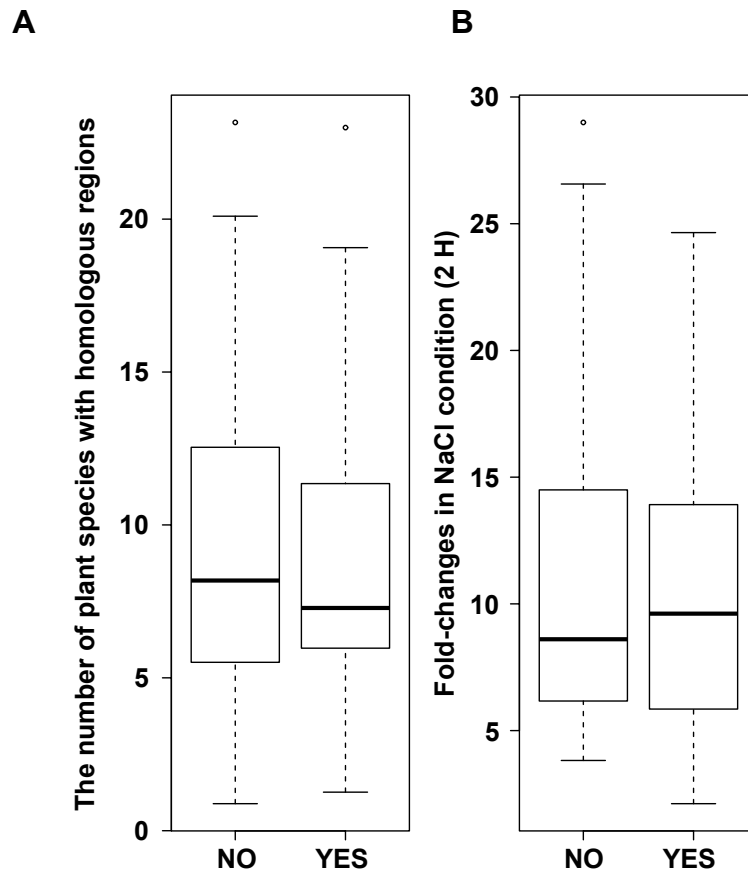
Fig. S13. High purity AT13 peptide induces salinity stress tolerance. Images of representative plants under a liquid culture-based salinity stress test. Seven-day-old plants were pre-treated with high purity (>90%) Pep3 or AT13(11-23) peptides for 3 days. Subsequently, 150 mM NaCl was applied with peptides under a liquid culture-based salinity stress test. Chlorophyll contents were measured to evaluate tolerance and sensitivity to the stress treatment.

Fig. S14. Detection of the AtPep3 peptide. (A) C-terminal amino acid sequence of AtPep3. Underline indicates the detected fragment of Pep3 by LC-MS/MS and red characters indicate the minimum size of the Pep3 peptide required to confer tolerance to salinity stress under our experimental conditions. (B) LC-MS/MS spectrum of the AtPep3 fragment. (C, D) Relative abundance of AtPep3 detected from non-treated (C) and NaCl treated samples (D).

Fig. S15. *pepr* mutants exhibited salt sensitive phenotype. Representative image of *pepr* mutant plants under liquid culture-based salinity stress test. Ten-day-old plants were treated with 125 mM NaCl and grown on 10 more days. Chlorophyll contents were measured to evaluate tolerance and sensitivity.

Fig. S16. Phenotype of *pepr* mutants plants subjected to salinity stress. Images of representative plants after each peptide/salt treatment (top). Chlorophyll content in plants after each peptide/salt treatment (bottom). Seven-day-old single or double receptor mutant plants were pre-treated with 100 nM of a Pep3 peptide fragment for 3 days (peptide pre-treatment) followed by a combined treatment with the peptide plus 150 mM NaCl. All treatments were carried out in liquid culture. Plants were grown for 10 days and chlorophyll content was subsequently assessed

Fig. S1



C

	No. of gene with secreted signal	No. of gene without secreted signal	Proportion of genes with secreted signal
No. of genes analyzed in the present study	8	9	47%
No. of genes not analyzed in the present study	11	34	24%

Fig. S2

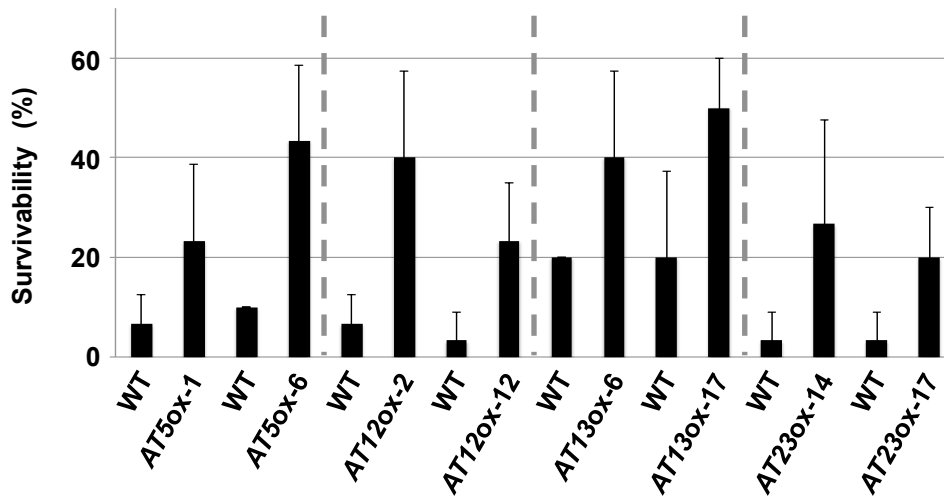
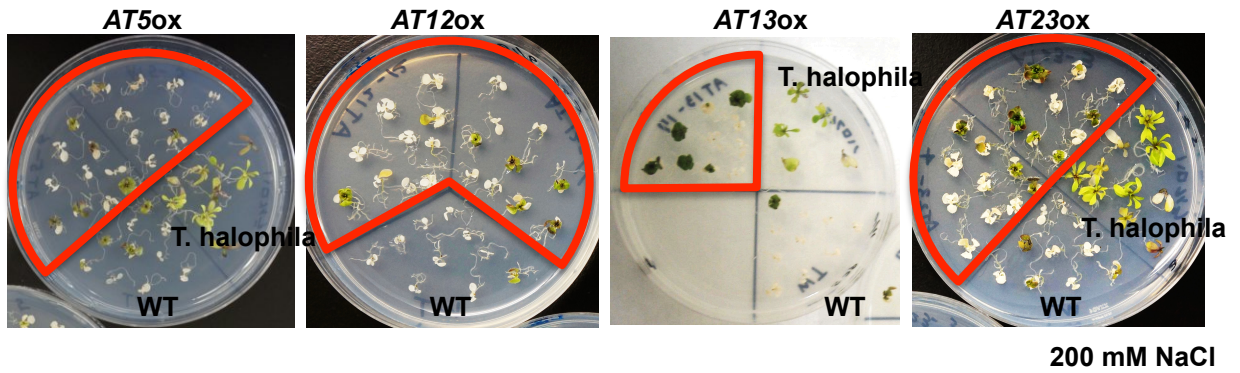
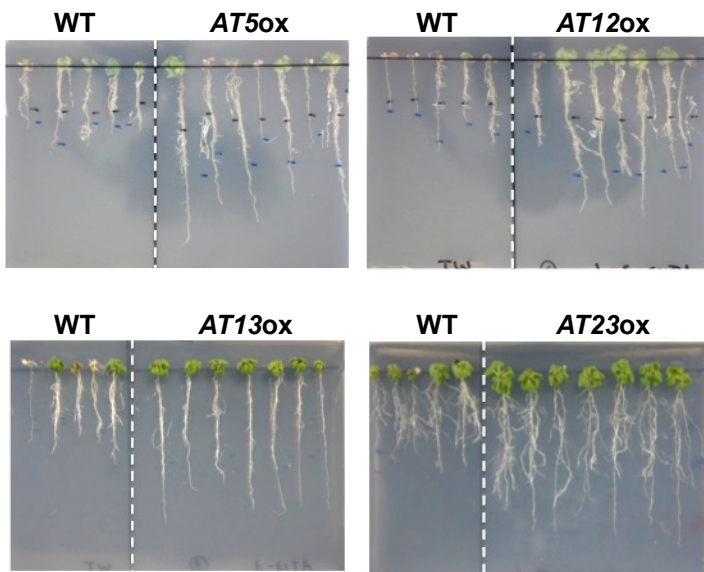


Fig. S3



150 mM NaCl

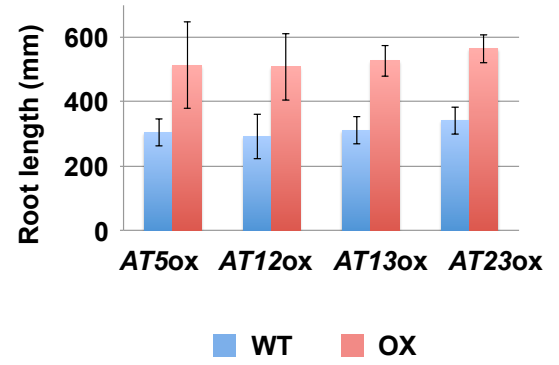


Fig. S4

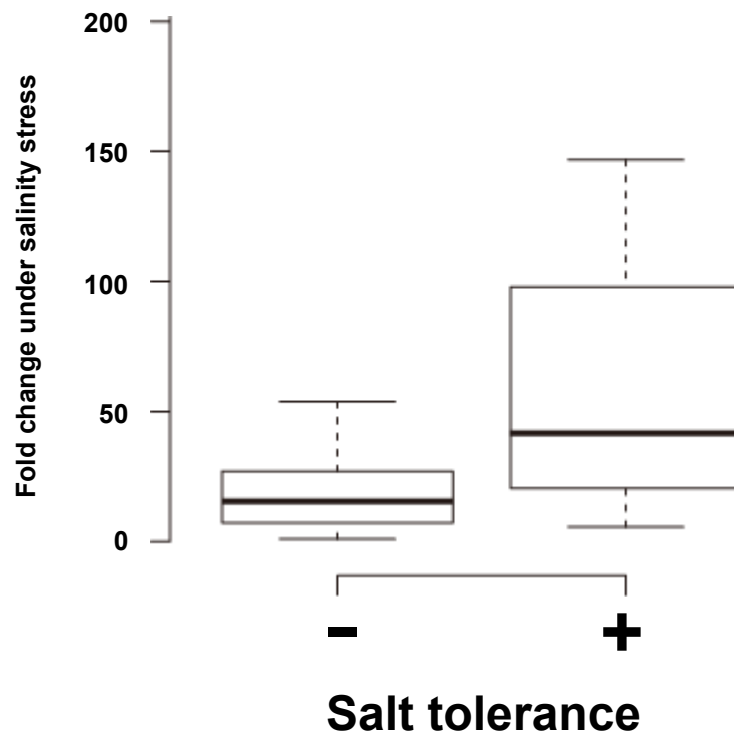


Fig. S5

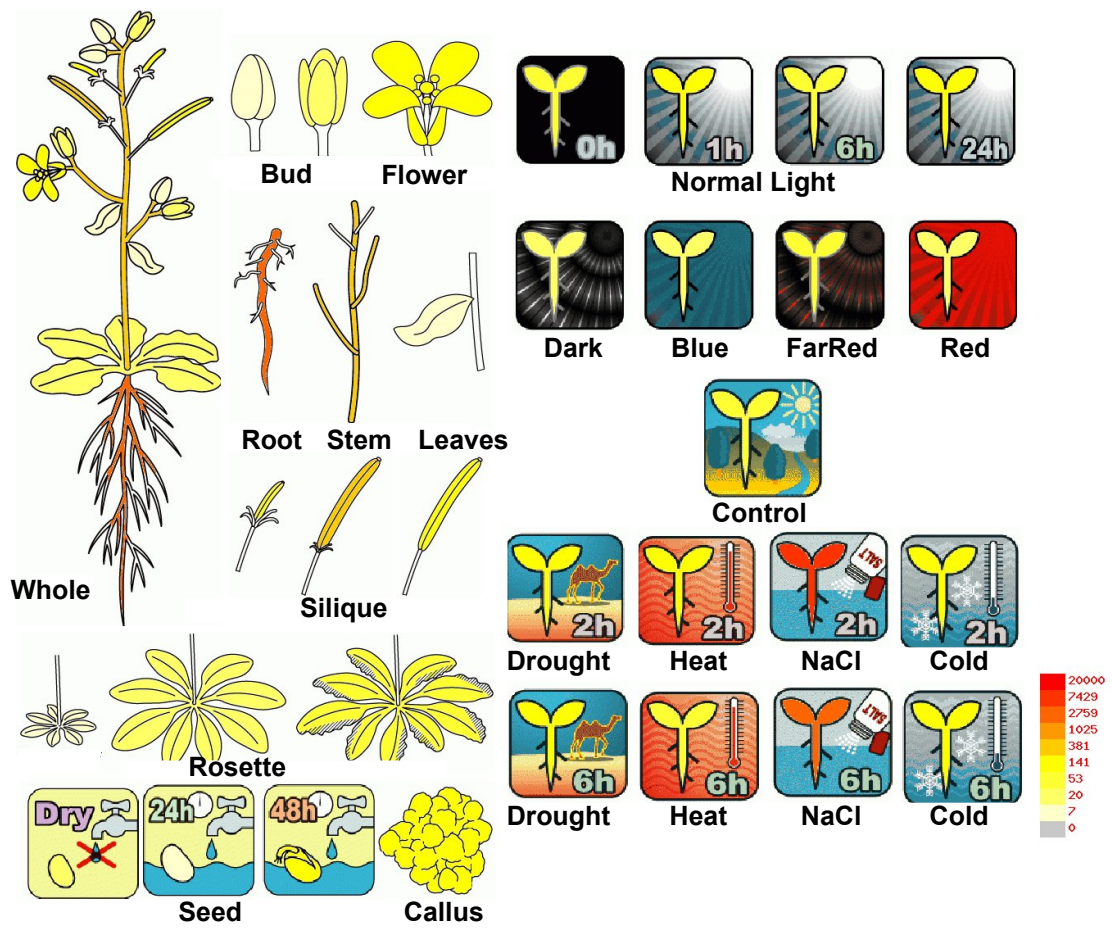
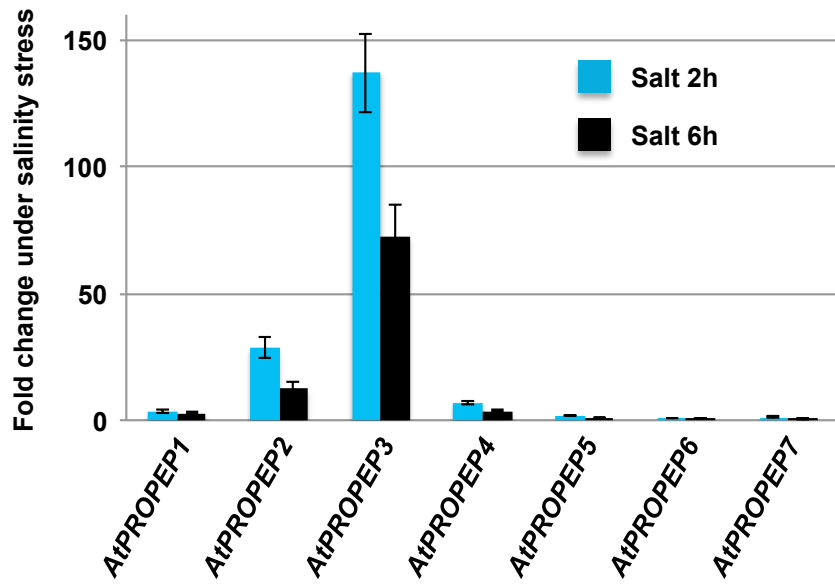


Fig. S6

A



B

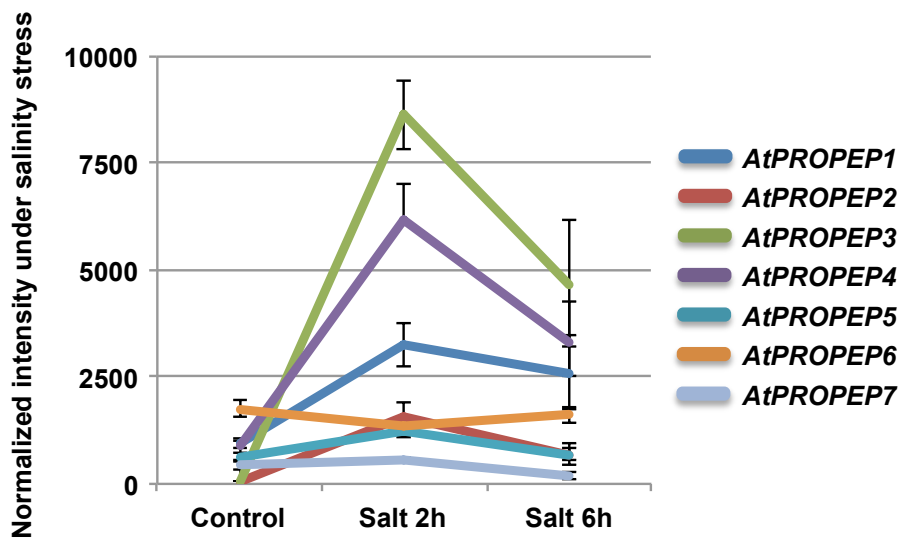


Fig. S7

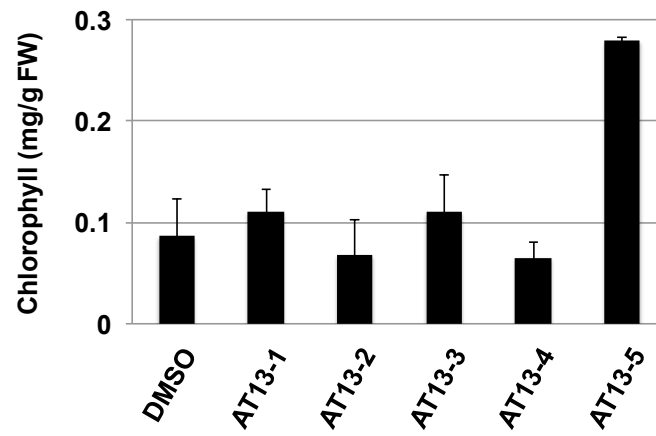
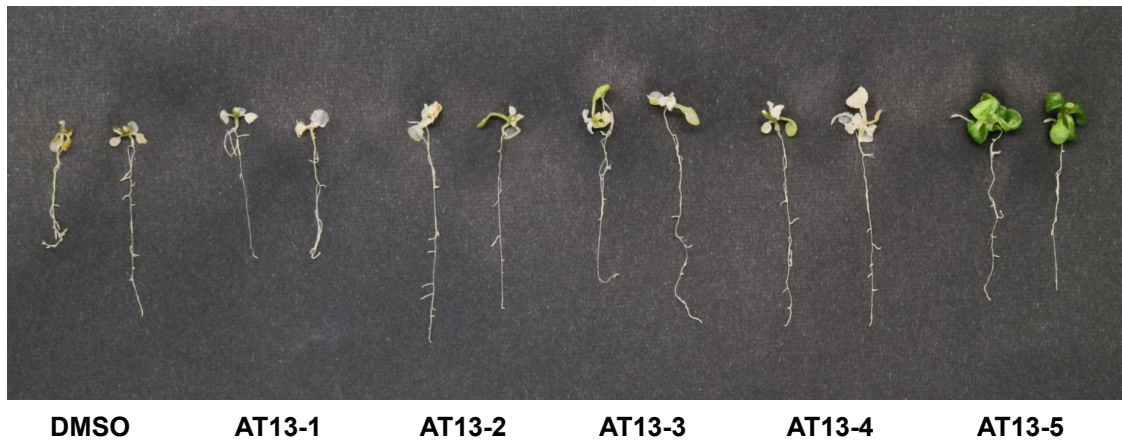


Fig. S8

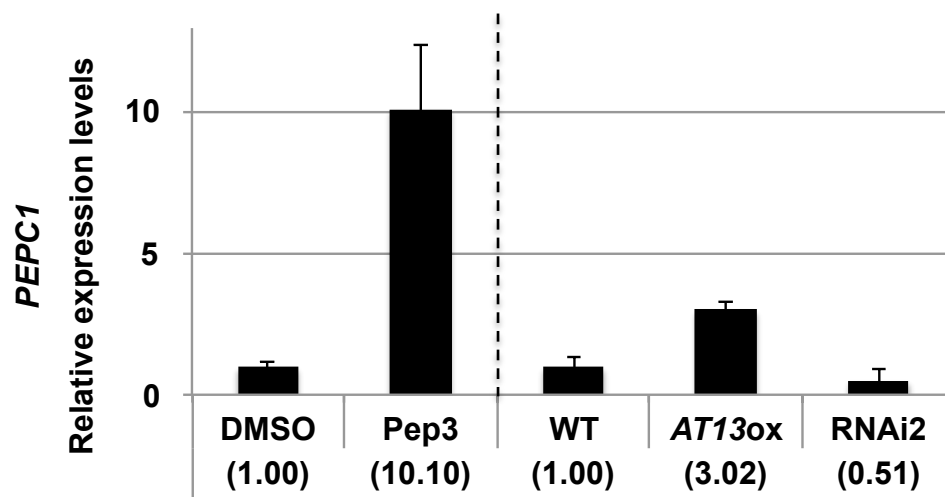


Fig. S9

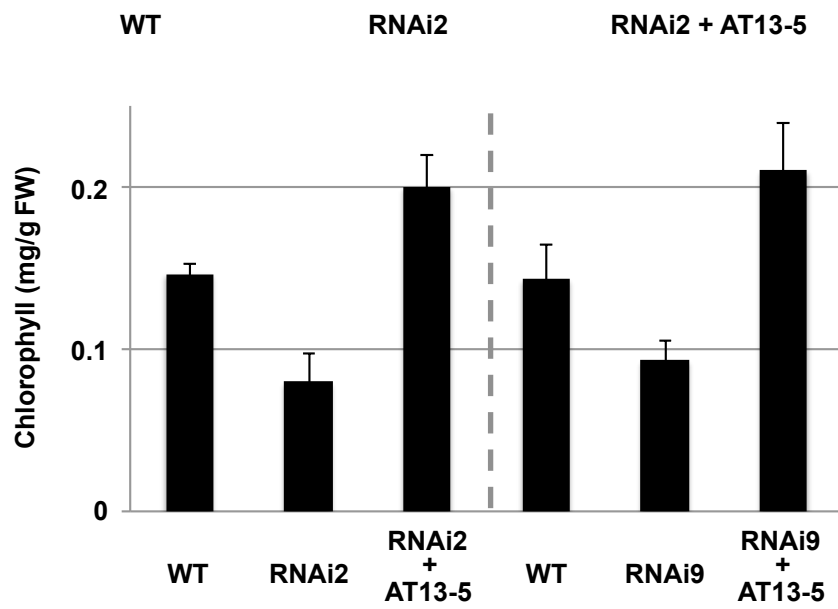


Fig. S10

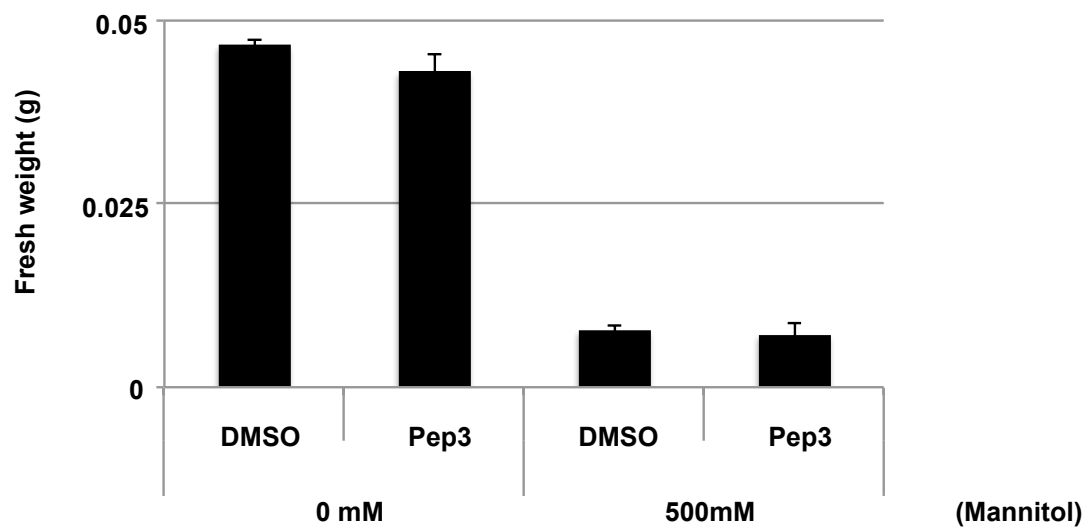


Fig. S11

-EEDGMTIEIKARGKNKTKPTPSSGKGGKHN*

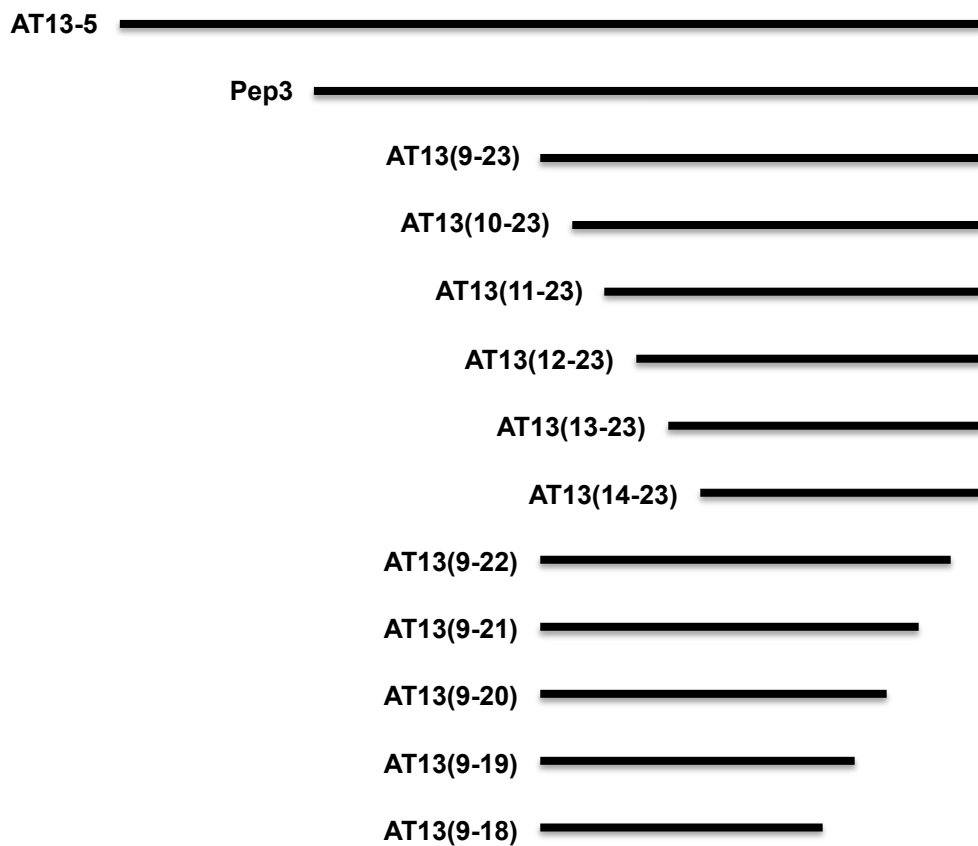


Fig. S12

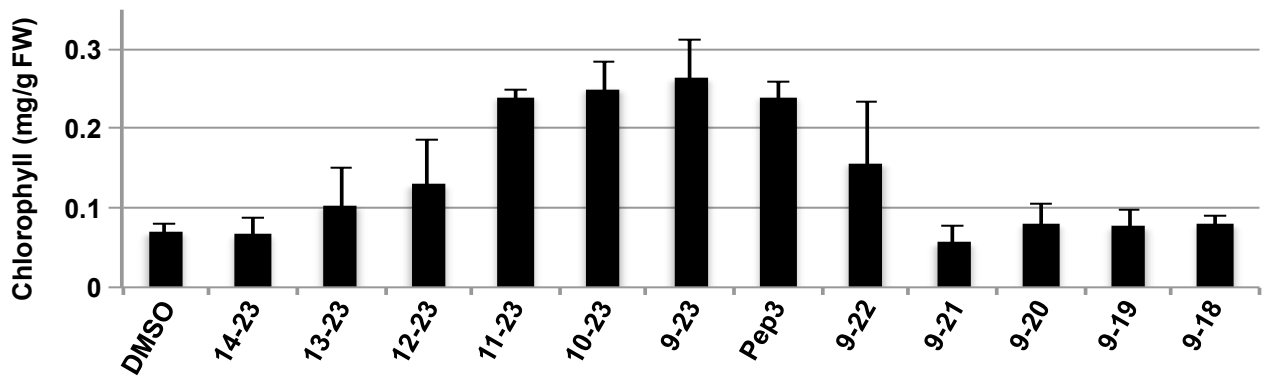
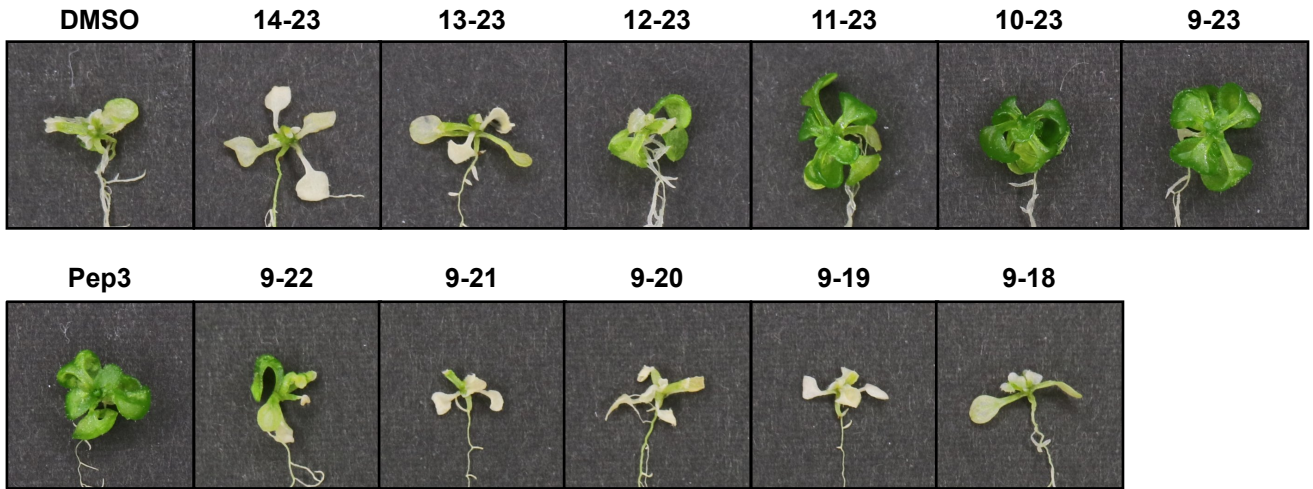


Fig. S13

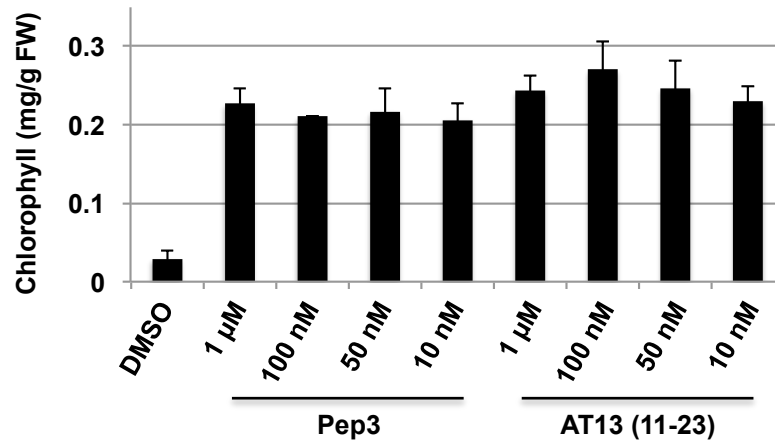
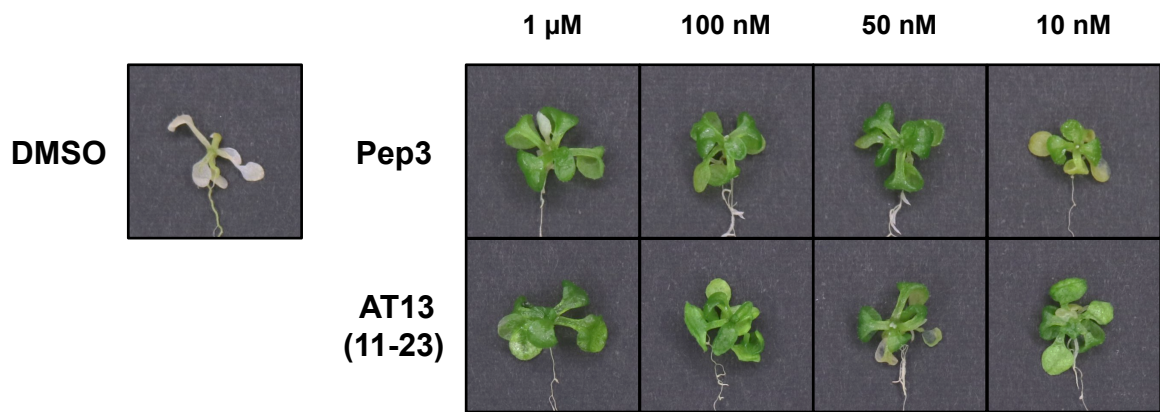
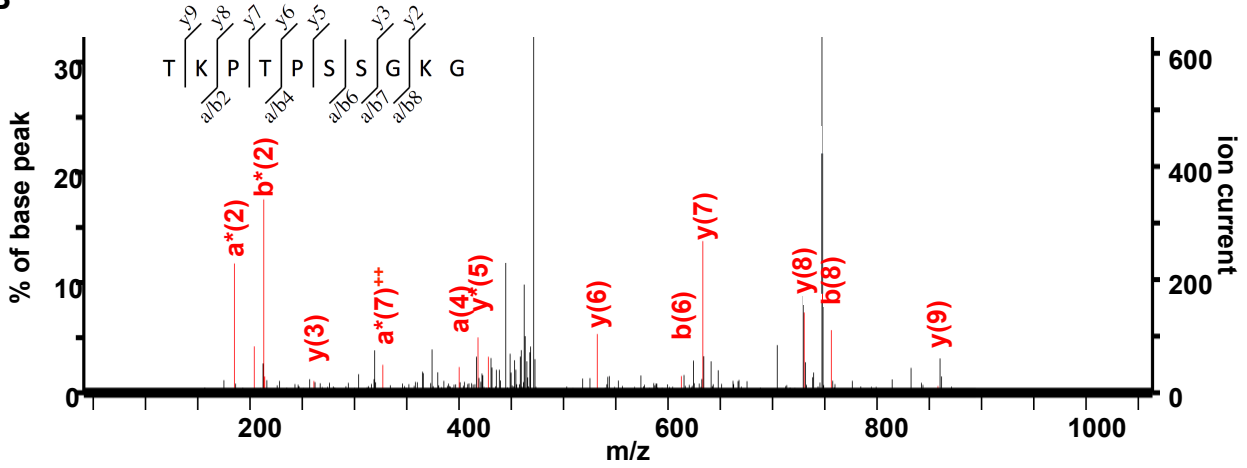


Fig. S14

A

AT13(Pep3) EIKARGKNKT**KTPSSGKG**GKHN*

B

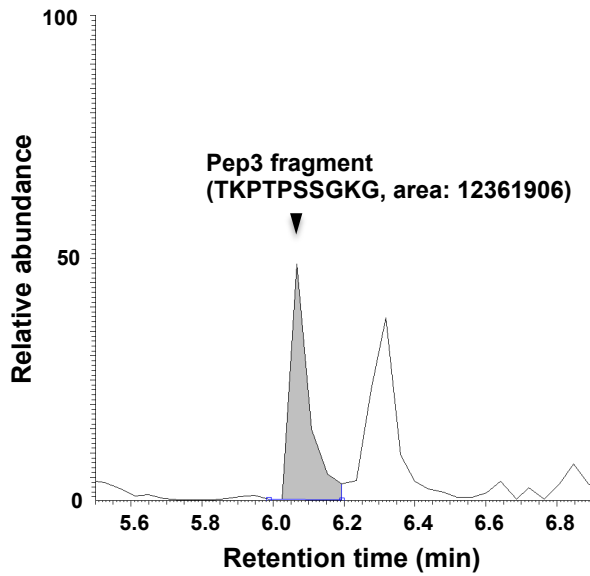


00072 Mass: 2419 Score: 19 Matches: 1(1) Sequences: 1(1) emPAI: 2.63

AT13(Pep3)

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
475	480.2611	958.5077	958.5084	-0.67	1	19	0.011	1	U	K.TKTPSSGKG.G

C



D

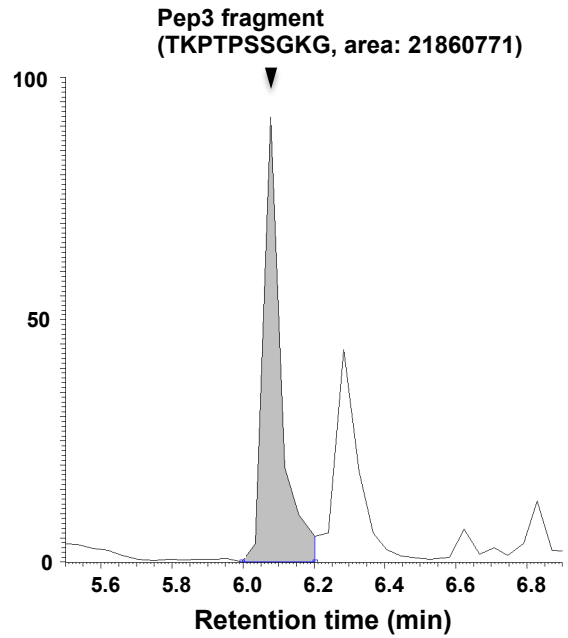
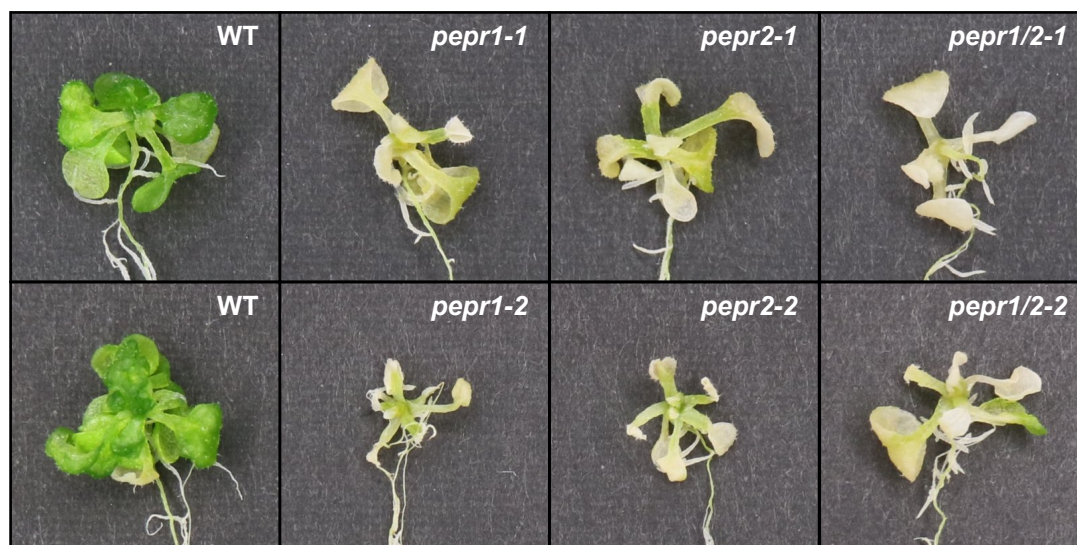


Fig. S15



125 mM NaCl

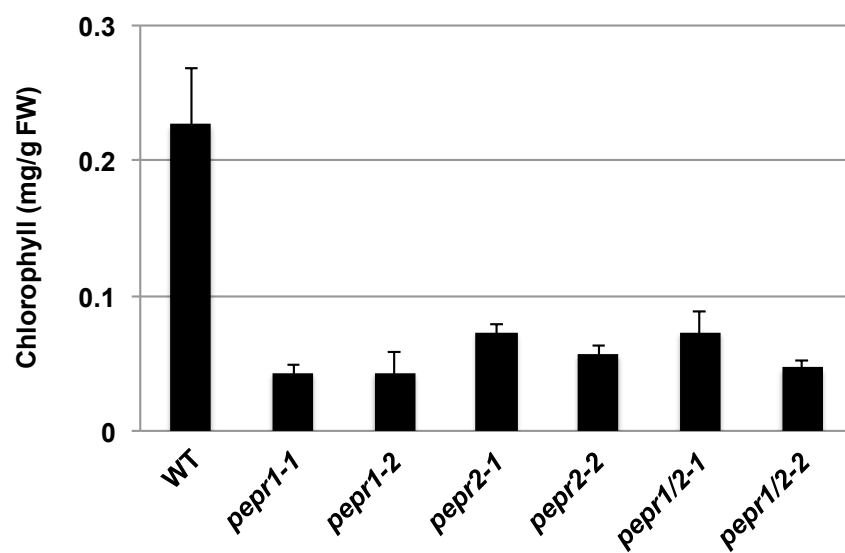


Fig. S16

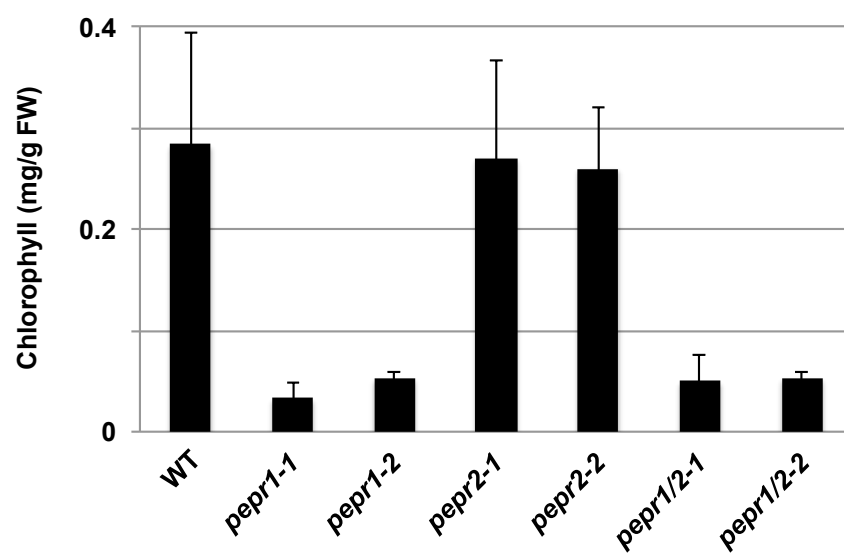
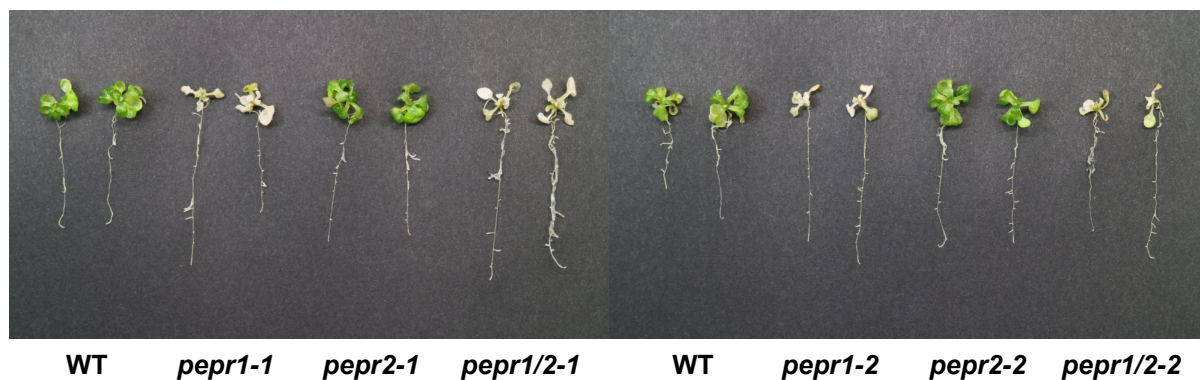


Table S1. Summary of up-regulated genes in NaCl treatment (2h or 6h)

Gene ID ^{a)}	Fold changes		Gene Description	Secreted signaling ^{b)}	The number of plant species with homolog ^{c)}	Transgenic plants
	NaCl 2h	NaCl 6h				
AT1G65510	6.9	7.3	similar to unknown protein	YES	6	NO
AT1G65500	5.5	17.7	similar to unknown protein	YES	6	NO
AT1G68765	6.5	22.2	IDA (INFLORESCENCE DEFICIENT IN ABSCISSION)	YES	8	NO
AT1G29640	7.3	16.5	similar to unknown protein	NO	15	YES
AT1G53903	15.4	26.6	senescence-associated protein-related	NO	7	NO
AT1G63245	11.3	6.1	CLE14 (CLAVATA3/ESR-RELATED 14)	YES	10	NO
AT1G61255	8.4	9.6	similar to glycine-rich protein	YES	5	YES
AT1G51920	9.1	15.6	unknown protein	YES	0	NO
AT1G05340	14.0	14.4	similar to unknown protein	NO	0	NO
AT1G07985	16.8	3.9	Expressed protein	NO	0	NO
AT1G06135	23.0	12.4	similar to unknown protein	YES	5	YES
AT1G53610	5.0	4.2	similar to unknown protein	YES	0	NO
AT1G15010	7.8	7.6	similar to unknown protein	NO	7	YES
AT1G64107	4.9	13.7	Encodes a defensin-like (DEFL) family protein.	YES	6	YES
AT1G64195	3.5	10.7	Encodes a defensin-like (DEFL) family protein.	YES	8	NO
AT1G67856	14.0	29.0	protein binding / zinc ion binding	NO	13	NO
AT2G22860	12.4	23.8	ATPSK2 (PHYTOSULFOKINE 2 PRECURSOR)	YES	5	YES
AT2G41730	15.9	11.5	similar to unknown protein	NO	6	NO
AT2G36440	5.8	15.2	unknown protein	NO	4	NO
AT2G21820	9.0	5.9	similar to hypothetical protein	NO	5	NO
AT2G44578	5.3	6.3	protein binding / zinc ion binding	NO	11	YES
AT2G36261	4.1	18.0	unknown protein	NO	0	NO
AT2G44581	6.1	13.9	protein binding / zinc ion binding	NO	12	YES
AT3G02480	6.5	7.3	ABA-responsive protein-related	NO	5	YES
AT3G23170	14.1	15.2	similar to unknown protein	NO	0	NO
AT3G10930	7.4	5.8	similar to unknown protein	YES	8	YES
AT3G53232	8.5	6.7	DVL20/RTFL1	NO	6	NO
AT3G60966	6.1	8.4	protein binding / zinc ion binding	NO	2	NO
AT3G03341	8.6	9.1	similar to Os01g0644200	NO	8	NO
AT3G15518	9.7	9.0	similar to hypothetical protein [Nicotiana benthamiana]	NO	6	NO
AT3G15534	7.3	9.9	similar to unknown protein	NO	8	NO
AT4G27530	20.1	10.7	similar to unknown protein	NO	6	NO
AT4G28703	12.5	7.2	similar to unknown protein	NO	0	NO
AT4G36500	23.2	11.1	similar to unknown protein	NO	5	NO
AT4G27654	6.1	5.1	unknown protein	NO	0	NO
AT4G21920	4.4	8.6	unknown protein	NO	0	NO
AT4G18980	17.9	6.2	similar to unknown protein	NO	9	NO
AT5G44575	2.1	13.1	unknown protein	NO	0	NO
AT5G01225	1.3	2.1	similar to unknown protein	NO	6	YES
AT5G46295	8.2	7.0	unknown protein	NO	0	NO
AT5G45630	9.5	7.6	similar to unknown protein	NO	2	NO
AT5G09980	11.2	4.8	PROPEP4	NO	0	NO
AT5G24640	10.6	14.8	similar to unknown protein	NO	4	NO
AT5G05300	7.0	24.6	similar to unknown protein	YES	6	YES
AT5G33355	0.9	4.5	Encodes a defensin-like (DEFL) family protein.	NO	8	NO
AT5G35110	12.5	7.0	similar to unknown protein	NO	0	NO
AT5G64905	19.1	5.6	PROPEP3	NO	4	YES
AT5G64890	8.0	4.3	PROPEP2	NO	4	NO
AT5G43570	4.5	8.4	serine-type endopeptidase inhibitor	NO	4	NO
AT5G19875	8.1	3.8	similar to unknown protein	YES	3	NO
AT5G36920	4.7	22.8	similar to unknown protein	YES	5	NO
AT5G47175	14.9	21.2	LCR3	YES	9	YES
AT5G54165	11.4	10.2	unknown protein	NO	8	YES
AT5G44585	4.4	5.8	unknown protein	YES	2	NO
sORF5958	7.1	7.6	Located on unannotated full length cDNA (RAFL04-20-O22)	NO	3	NO
sORF7775	3.2	4.0	Located on unannotated full length cDNA (RAFL21-83-H09)	NO	7	YES
sORF4875	6.0	2.8	chr3 + 20317641-20317931	YES	6	YES
sORF0625	15.2	9.1	chr1 + 11440203-11440469	NO	0	NO
sORF0541	7.3	6.6	chr1 + 9952787-9952909	NO	0	NO
sORF7215	5.5	5.7	chr5 + 14889303-14889482	NO	0	NO
sORF6740	16.2	14.5	chr5 - 9156935-9157072	NO	2	NO
sORF7726	5.1	21.7	Located on unannotated full length cDNA (EF182830)	YES	3	NO
AT1G71000	4.1	54.7	DNAJ heat shock N-terminal domain-containing protein			
AT1G28480	24.5	11.4	GRX480; thiol-disulfide exchange intermediate			
AT1G45145	13.8	9.7	ATTRX5 (thioredoxin H-type 5); thiol-disulfide exchange intermediate			
AT1G55780	5.6	9.7	heavy-metal-associated domain-containing protein			
AT1G32560	9.1	8.4	late embryogenesis abundant group 1 domain-containing protein			

AT2G42530	6.5	5.9	COR15B, a protein that protects chloroplast membranes during freezing.			
AT2G41280	9.7	3.8	LEA (Late Embryogenesis Abundant) proteins			
AT2G40170	6.5	11.9	ATEM6/GEA6 (ARABIDOPSIS EARLY METHIONINE-LABELLED 6)			
AT2G43510	3.7	15.5	ATT11 (ARABIDOPSIS THALIANA TRYPSIN INHIBITOR PROTEIN 1)			
AT2G35730	10.4	11.3	heavy-metal-associated domain-containing protein			
AT2G37870	15.3	17.7	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein			
AT3G21890	19.8	2.6	zinc finger (B-box type) family protein			
AT3G18250	4.6	4.2	contains domain PROKAR LIPOPROTEIN (PS51257)			
AT4G33040	9.1	6.0	glutaredoxin family protein			
AT5G13080	7.7	7.7	WRKY75 (WRKY DNA-BINDING PROTEIN 75); transcription factor			
AT5G59310	8.3	14.4	LTP4 (LIPID TRANSFER PROTEIN 4); lipid binding			
AT5G59320	7.1	14.6	LTP3 (LIPID TRANSFER PROTEIN 3); lipid binding			
AT5G43580	10.7	8.3	serine-type endopeptidase inhibitor			
AT5G05365	5.0	6.8	metal ion binding			

a) Gene ID registered in The Arabidopsis Information Resource (TAIR, <https://www.arabidopsis.org/>) and our previous paper (Hanada et al., PNAS 2013)

b) Secreted signal by Signal IP software (Petersen et al., Nature Methods, 8:785-786, 2011)

c) The following genomes were used to assess conservation of small coding genes: *Physcomitrella patens* (http://genome.jgi-psf.org/Phypa1_1/), *Selaginella moellendorffii* (<http://genome.jgi-psf.org/Selmo1/>), *Zea mays* (<http://www.maizegenome.org/>), *Sorghum bicolor* (<http://genome.jgi-psf.org/Sorbi1/Sorbi1.home.html>), *Brachypodium distachyon* (<http://www.jgi.doe.gov/>), *Oryza sativa* (<http://rapdb.dna.affrc.go.jp/download/>), *Mimulus guttatus* (<http://genome.jgi-psf.org/mimulus/>), *Vitis vinifera* (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>), *Ricinus communis* (<http://castorbean.jcvi.org/downloads.php>), *Manihot esculenta* (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Mesculenta), *Populus trichocarpa* (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Ptrichocarpa), *Cucumis sativus* (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Csativus/), *Glycine max* (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v4.0/Gmax), *Medicago truncatula* (<http://www.medicago.org/genome/downloads/Mt3/>), *Carica papaya* (<ftp://asgpb.hawaii.edu/papaya/annotation/>) and *Arabidopsis lyrata* (<http://genome.jgi-psf.org/Araly1/>). Sequence pairs were regarded as homologous sequences if they had less than 1 e-value by BLAST search (tblastn)

Table S2. Results of the initial screening of AT-ox plants using a plate-based salinity stress test

Gene ID	Line number	Survival ratio ox/WT	Tolerance ^{a)}	Gene ID	Line number	Survival ratio ox/WT	Tolerance ^{a)}
AT4 (SORF7775)	1	1.25		AT23 (AT1G06135)	1	0.33	
	2	1.25			2	0.67	
	3	1.17			3	1.78	
	4	0.83			4	1.50	
	5	1.33			5	0.80	
	6	2.00	+		6	0.60	
	7	2.50	+		7	1.00	
	8	1.00			8	2.00	+
	9	1.20			9	0.63	
	10	1.80			10	0.50	
	11	1.40			11	1.00	
	12	1.20			12	1.50	
	13	1.00			13	1.33	
	14	0.67			14	8.00	+
	15	1.50			15	0.63	
	16	1.50			16	0.75	
	17	1.50			17	6.00	+
AT5 (AT5G05300)	1	3.50	+	AT32 (AT1G64107)	1	-	
	2	0.00			2	-	
	3	N.A.			3	-	
	4	1.33			4	-	
	5	2.33	+		5	-	
	6	4.33	+		6	-	
	7	2.00	+		7	0.50	
	8	0.60			8	0.25	
	9	0.36			9	0.00	
	10	0.55			10	0.83	
	11	0.56			11	0.00	
	12	1.40			12	0.40	
	13	0.00			13	0.00	
	14	1.63			14	0.00	
	15	2.67	+		15	2.50	+
	16	1.67			16	1.50	
	17	1.22			17	0.67	
AT10 (AT2G22860)	1	1.25		AT33 (AT5G01225)	1	0.70	
	2	2.00	+		2	1.10	
	3	1.67			3	0.50	
	4	2.00	+		4	0.30	
	5	1.25			5	0.44	
	6	0.88			6	0.63	
	7	1.00			7	0.50	
	8	1.17			8	0.75	
	9	1.00			9	0.00	
	10	0.00			10	0.31	
	11	0.44			11	0.69	
	12	0.56			12	0.50	
	13	1.11			13	0.50	
	14	1.00			14	0.25	
	15	0.20			15	1.00	
	16	0.20			16	0.50	
	17	0.88			17	3.00	+
AT11 (AT2G44581)	1	1.18		AT35 (AT5G54165)	1	5.00	+
	2	1.18			2	5.00	+
	3	2.20	+		3	1.45	
	4	0.40			4	1.00	
	5	1.00			5	1.44	
	6	1.00			6	1.17	
	7	0.75			7	3.00	+
	8	0.50			8	2.50	+
	9	0.57			9	2.33	+
	10	1.14			10	4.33	+
	11	9.00	+		11	1.00	
	12	1.00			12	0.00	
	13	1.67			13	0.67	
	14	1.00			14	0.00	
	15	0.83			15	0.75	

	16	0.83			16	0.56	
	17	0.17			17	1.11	
AT12 (AT2G44578)	1	4.00	+	AT47 (AT3G02480)	1	0.75	
	2	6.00	+		2	1.13	
	3	N.A.			3	1.00	
	4	N.A.			4	1.00	
	5	1.50			5	1.44	
	6	1.33			6	0.78	
	7	0.50			7	-	
	8	0.75			8	-	
	9	1.67			9	1.13	
	10	1.50			10	1.13	
	11	3.00	+		11	2.00	+
	12	7.00	+		12	0.00	
	13	0.67			13	1.00	
	14	0.33			14	0.40	
	15	1.75			15	0.80	
	16	0.71			16	0.30	
	17	2.50	+		17	0.83	
AT13 (AT5G64905)	1	1.17		AT56 (AT1G15010)	1	4.50	+
	2	1.00			2	1.50	
	3	1.00			3	9.00	+
	4	0.00			4	5.00	+
	5	0.00			5	N.A.	
	6	2.00	+		6	N.A.	
	7	1.50			7	0.63	
	8	0.50			8	0.75	
	9	1.00			9	6.50	+
	10	0.43			10	3.50	+
	11	1.33			11	7.00	+
	12	0.83			12	4.50	+
	13	1.22			13	1.50	
	14	0.78			14	4.50	+
	15	1.67			15	1.75	
	16	1.67			16	1.25	
	17	2.50	+		17	1.13	
AT18 (AT1G29640)	1	1.25		AT68 (AT3G10930)	1	0.40	
	2	1.50			2	0.93	
	3	1.00			3	2.80	+
	4	0.25			4	2.00	+
	5	3.50	+		5	0.78	
	6	0.50			6	1.11	
	7	0.67			7	0.58	
	8	1.33			8	0.33	
	9	0.00			9	1.00	
	10	0.00			10	1.75	
	11	0.67			11	1.67	
	12	0.67			12	1.50	
	13	-			13	1.50	
	14	-			14	2.00	+
	15	-			15	0.75	
	16	-			16	0.91	
	17	-			17	2.00	+
AT19 (AT1G61255)	1	-		sORF4875	1	0.70	
	2	1.75			2	0.70	
	3	0.67			3	2.00	+
	4	-			4	1.33	
	5	3.50	+		5	0.73	
	6	3.50	+		6	0.55	
	7	5.00	+		7	0.94	
	8	2.00	+		8	0.59	
	9	7.00	+		9	1.30	
	10	5.00	+		10	1.60	
	11	1.13			11	0.64	
	12	1.25			12	1.00	
	13	2.00	+		13	1.70	
	14	2.25	+		14	1.90	
	15	1.73			15	1.00	
	16	0.75			16	1.50	

	17	0.50		17	2.00	+
AT20 (AT5G47175)	1	1.13				
	2	2.13	+			
	3	0.38				
	4	0.38				
	5	1.00				
	6	0.67				
	7	1.13				
	8	1.63				
	9	0.73				
	10	0.40				
	11	0.58				
	12	1.08				
	13	0.63				
	14	1.38				
	15	-				
	16	-				
	17	2.60	+			

a) "tolerance", "+" indicates salinity stress tolerant line by this test, which showed "survival ratio ox/WT" > 2
 "-" indicates not tested and "N.A." indicates survival proportion of WT was "0".

Table S3. Chemically synthesized peptide fragments of AtPep3/AT13 peptide

Peptide Name	Sequence: NH ₂ -Sequence-COOH	Purity analysed by HPLC (%)	M.W. (theoretical)	M.W. detected by MALDI-TOF MS	Isoelectric point	Average hydrophobicity (Kyte/Doolittle)	Charge Density
AT13-1	MENLRNGEDNGSLIPFTFFDQSSVTIPLLK	91.52	3383.78	3384.48	4.1	-0.18	0.27
AT13-2	FTFFDQSSVTIPLLKCSGLESSSSSSSSCD	61.82	3147.40	3148.81	3.9	0.11	0.20
AT13-3	CSGLESSSSSSSSCDLSSSHSEEDSIDIK	53.95	3080.09	3082.01	3.7	-0.72	0.33
AT13-4	LSSSHSEEDSIDIKEEEEEEEEEDGMTIEI	60.66	3468.48	3467.11	3.4	-1.37	0.60
AT13-5	EEDGMTIEIKARGKNKTKPTSSGKGGKHN	68.97	3196.55	3196.09	10.5	-1.61	0.43
Pep3	EIKARGKNKTKPTSSGKGGKHN	55.56	2420.73	2421.07	11.5	-1.87	0.43
AT13(9-23)	KTKPTSSGKGGKHN	58.63	1523.69	1523.85	11.3	-1.98	0.40
AT13(10-23)	TKPTSSGKGGKHN	81.19	1395.52	1395.80	11.1	-1.84	0.36
AT13(11-23)	KPTSSGKGGKHN	62.17	1294.42	1294.60	11.1	-1.93	0.38
AT13(12-23)	PTSSGKGGKHN	84.58	1166.24	1166.20	10.8	-1.77	0.33
AT13(13-23)	TPSSGKGGKHN	69.28	1069.13	1069.40	10.8	-1.78	0.36
AT13(14-23)	TPSSGKGGKHN	50.36	968.03	968.00	10.8	-1.89	0.40
AT13(9-22)	KTKPTSSGKGGKH	58.67	1409.59	1409.70	11.3	-1.87	0.43
AT13(9-21)	KTKPTSSGKGGK	52.23	1272.45	1272.50	11.3	-1.77	0.46
AT13(9-20)	KTKPTSSGKGG	59.91	1144.28	1144.20	11.1	-1.59	0.42
AT13(9-19)	KTKPTSSGK	70.16	1087.23	1087.20	11.1	-1.70	0.45
AT13(9-18)	KTKPTSSGK	78.88	1030.18	1030.20	11.1	-1.83	0.50
Pep3-90%	EIKARGKNKTKPTSSGKGGKHN	95.96	2420.73	2421.37	11.5	-1.87	0.43
AT13(11-23)-90%	KPTSSGKGGKHN	99.04	1294.42	1294.89	11.1	-1.93	0.38

Table S4. Up/Down-regulated genes in both AtPROPEP3/AT13-ox plants and AtPep3/AT13 peptide treated plants

AGF [®] , Full length cDNA ID [®] and sORF ID [®]	Description	DMSO			AT13pep			AT13pep / DMSO	WT			AT13ox			AT13ox /WT
		rep1	rep2	rep3	rep1	rep2	rep3		rep1	rep2	rep3	rep1	rep2	rep3	
		AT1G03010	Phototropic-responsive NPH3 family protein	133.03	126.62	113.62	197.62		190.34	184.54	1.5	147.65	143.12	125.61	
AT1G03020	Thioredoxin superfamily protein	50.457	28.88	37.25	217.58	253.59	266.56	6.3	53.978	52.455	53.571	296.85	304.54	325.6	5.8
AT1G03400	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	93.856	106.42	114.94	164.36	160.51	169.1	1.6	137.23	130.22	136.71	184.74	187.65	191.52	1.4
AT1G03820	unknown protein	634.81	604.05	692.32	1399.4	1485.8	1316.1	2.2	765.33	845.63	810.32	588.62	549.98	617.99	0.7
AT1G05560	UDP-glycosyltransferase 75B1	177.93	172.88	180.67	67.786	87.244	66.658	0.4	346.95	433.28	386.83	220.77	210.56	230.75	0.6
AT1G08310	alpha/beta-Hydrolases superfamily protein	101.2	93.428	90.656	229.08	253.87	204.55	2.4	147.57	125.86	124.61	372.31	405.93	363.7	2.9
AT1G10585	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	364.24	452.48	388.34	170.92	162.05	168.57	0.4	342.52	333.05	327.06	245.24	216.59	252.61	0.7
AT1G10640	Pectin lyase-like superfamily protein	422.09	440.85	402.9	256.92	285.76	252.6	0.6	602.43	631.13	575.68	480.96	491.62	503.95	0.8
AT1G11740	ankyrin repeat family protein	1587.1	1538.5	1656.9	1237.6	1277.5	1169.4	0.8	1283.6	1358.9	1343.9	2214.8	1812.4	2284.6	1.6
AT1G13420	sulfotransferase 4B	704.41	647.82	723.01	354.33	419.48	361.74	0.5	465.6	390.27	509.14	708.58	745.14	787.49	1.6
AT1G14220	Ribonuclease T2 family protein	469.77	542.51	506.67	286.37	294.63	236.1	0.5	384.82	348.23	404.18	526.9	517.86	548.73	1.4
AT1G14250	GDA1/CD39 nucleoside phosphatase family protein	12.242	12.683	13.653	115.87	104.13	116.07	8.7	23.973	22.946	31.146	57.9	70.795	79.694	2.7
AT1G17710	Pyridoxal phosphate phosphatase-related protein	50.707	40.057	43.953	546.77	481.78	482.78	11.2	236.28	151.66	224.08	848.48	725.96	784.61	3.9
AT1G18200	RAB GTPase homolog A6B	428.82	429.5	432.44	610.59	562.96	584.1	1.4	654.56	631.37	610.18	802.27	733.59	767.02	1.2
AT1G20300	Pentatricopeptide repeat (PPR) superfamily protein	294.43	303.34	296.91	265.26	264.99	271	0.9	485.31	503.74	503.83	312.56	364.15	374.88	0.7
AT1G21460	Nodulin MtN3 family protein	1474.5	1324.5	1396.6	2222.1	2239.7	2010.3	1.5	882.28	865.06	850.34	1277.1	1294.5	1317.9	1.5
AT1G23060	unknown protein	317.1	334.02	303.18	240.78	243.09	229.35	0.7	284.59	280.74	259.7	344.47	326.37	344.47	1.2
AT1G25560	AP2/B3 transcription factor family protein	20980	19819	19179	29889	28203	30052	1.5	22348	20161	20658	26832	25805	24616	1.2
AT1G26730	EXS (ERD1/XPR1/SYG1) family protein	178.43	174.08	178.6	293.97	299.96	316.94	1.7	292.31	276.26	293.19	348.35	332.09	360.48	1.2
AT1G26930	Galactose oxidase/kelch repeat superfamily protein	3483.5	3632.8	3573.9	2831.3	2759.7	2856	0.8	3560.3	3932.1	3730.7	2688	2810.1	2861.4	0.7
AT1G31550	GDSSL-like Lipase/Acylhydrolase superfamily protein	242.2	254.85	275.89	361.62	361.31	342.73	1.4	218.64	186.03	238.72	313.73	362.38	354.62	1.6
AT1G35183	unknown protein	32.488	35.553	32.825	50.629	46.449	48.104	1.4	21.599	20.855	21.033	36.64	32.538	29.823	1.6
AT1G48260	CBL-interacting protein kinase 17	144.72	152.41	152.43	277.01	310.18	266.19	1.9	184.74	192.22	178.44	243.65	229.45	234.82	1.3
AT1G52690	Late embryogenesis abundant protein (LEA) family protein	619.79	660.61	527.9	91.9	128.97	54.369	0.2	306.55	335.65	251.03	135.79	122.71	124.7	0.4
AT1G54210	Ubiquitin-like superfamily protein	406.64	410.38	428.01	533.18	534.98	559.1	1.3	570.74	499.22	516.54	792.91	754.06	753.49	1.5
AT1G55740	seed imbibition 1	291.28	303.89	264.19	161.22	153.78	143.25	0.5	219.6	232.07	211.27	258.21	265.11	264.71	1.2
AT1G58420	Uncharacterised conserved protein UCP031279	117.23	145.83	138.62	288.63	253.7	275.52	2.0	135.26	127.61	125.63	160.81	187.1	162.46	1.3
AT1G59730	thioredoxin H-type 7	135.68	113.69	128.19	52.282	53.925	53.617	0.4	133.46	108.46	115.69	167.62	168.76	151.19	1.4
AT1G62500	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	1431.4	1616.3	1574.3	2000.8	2101.1	2088.6	1.3	2007.3	2022.8	2167.2	1508.9	1532	1765	0.8
AT1G63057	unknown protein	133.51	124.22	128.26	80.567	78.51	60.905	0.6	116.68	113.07	91.474	199.45	209.13	186.86	1.9
AT1G64170	cation/H+ exchanger 16	480.42	510.2	438.73	788.15	701.94	795.67	1.6	567.03	537.47	570.38	607.93	636.38	624	1.1
AT1G64750	deletion of SUV3 suppressor 1(I)	25400	24907	24833	23306	23089	22935	0.9	21783	20658	20817	24361	25819	25196	1.2
AT1G67600	Acid phosphatase/vanadium-dependent haloperoxidase-related protein	102.48	89.093	115.46	183.13	184.64	187.74	1.8	94.296	81.288	88.886	202.46	199.82	216.02	2.3
AT1G68740	EXS (ERD1/XPR1/SYG1) family protein	182.79	152.67	133.75	512.85	414.79	461.34	3.0	323.34	238	323.15	629.68	467.36	587.44	1.9
AT1G68825	ROTUNDIFOLIA like 15	216.05	219.13	230.05	94.434	89.874	90.285	0.4	202.57	207.72	212.12	239.44	236.57	235.05	1.1
AT1G72990	beta-galactosidase 17	462.21	463.01	452.32	508.22	527.59	510.81	1.1	492.35	479.58	463.25	544.14	531.14	545.6	1.1
AT1G80450	VQ motif-containing protein	51.667	57.989	55.918	82.166	80.472	81.056	1.5	95.69	100.72	104.95	67.752	82.378	65.773	0.7
AT2G14900	Gibberellin-regulated family protein	860.96	842.63	924.8	1487.8	1394.9	1362.6	1.6	750.25	733	787.86	1062.3	1025.4	1060.8	1.4
AT2G16700	actin depolymerizing factor 5	4937	4998.9	4968.9	5638.5	5596	5579.1	1.1	7198.7	6940.9	6795.2	5372.3	4702.5	5379.9	0.7
AT2G18730	diacylglycerol kinase 3	1093.8	1081.1	1134.2	1364.9	1392.2	1395.5	1.3	1282.4	1138.9	1263.6	1584.9	1633.1	1651.5	1.3
AT2G20750	expansin B1	152.77	151.01	153.74	80.838	86.809	67.473	0.5	149.58	124.24	150.18	83.692	99.8	94.381	0.7
AT2G20780	Major facilitator superfamily protein	3533.1	3584.8	3630.6	3851.3	3810.8	3821.1	1.1	3081	2951.3	3162.7	3464.8	3615.2	3557.1	1.2
AT2G21560	unknown protein	1083.5	1168.7	1141.6	580.86	607.53	580.86	0.5	1524.4	1408.4	1592.3	740.53	760.38	749.15	0.5
AT2G23840	HNH endonuclease	524.15	496.45	496.96	596.81	609.16	605.57	1.2	1047.7	1124	1100.6	1286.6	1376.6	1498	1.3
AT2G28430	unknown protein	4553.2	4667.3	4731.7	5106.8	5123.4	5101.3	1.1	3899.6	3933.6	3612.7	4876.7	5253.5	4552.1	1.3
AT2G28660	Chloroplast-targeted copper chaperone protein	411.93	394.42	422.25	589.48	548.27	608.14	1.4	478.66	415.43	464.45	549.98	564.36	591.32	1.3
AT2G29180	unknown protein	3941	4132.5	3957.8	3179.4	3342	3211	0.8	3334.1	3344.1	3434.7	4283.9	4566.1	4678.9	1.3
AT2G29310	NAD(P)-binding Rossmann-fold superfamily protein	3759.5	3721.8	4097	5062.8	5304.6	5543.7	1.4	4189	4073.2	4880.6	5938.3	5898.3	6204.9	1.4

AT2G29490	glutathione S-transferase TAU 1	532.88	588.57	564.63	265.6	252.07	275.4	0.5	616.5	670.86	597.18	442.7	412.09	485.89	0.7
AT2G31750	UDP-glucosyl transferase 74D1	638.93	665.77	670	485.08	490.99	538.45	0.8	1002.7	1098.9	1050.3	806.35	824.71	880.65	0.8
AT2G34210	Transcription elongation factor Spt5	2.8952	4.7498	2.038	28.393	23.958	27.515	8.2	8.8881	7.0153	7.5104	43.282	37.599	49.476	5.6
AT2G37460	nodulin MtN21 /EamA-like transporter family protein	479.27	608.39	470.08	960.71	1006.6	976.62	1.9	809.47	810.25	871.67	482.94	480.02	486.44	0.6
AT2G39140	pseudouridine synthase family protein	83.967	88.312	83.564	70.49	71.244	71.273	0.8	151.77	179.54	152.86	95.984	111.08	113.67	0.7
AT2G39700	expansin A4	1328.7	1346.7	1471.9	2254.2	2556.8	2396.4	1.7	1581.2	1619.4	1565.2	1225	1347.9	1359.1	0.8
AT2G40390	unknown protein	30.377	32.268	33.434	20.228	21.883	19.226	0.6	49.348	42.437	50.801	21.599	24.947	26.949	0.5
AT2G40940	ethylene response sensor 1	4147	4413	4337.6	5569.7	5238.4	5618.8	1.3	5823.7	5805.2	5914.3	5076.4	4965.5	5131.2	0.9
AT2G41730	unknown protein	1028	1200.4	1120	2171.6	2179.9	2412.7	2.0	3446.8	3338.8	3895.4	4936.7	4843.9	5299	1.4
AT2G42840	protodermal factor 1	11389	11123	11855	7700.8	8328.1	7822.6	0.7	12519	14145	13334	8050.3	9060.6	8124.5	0.6
AT2G45130	SPX domain gene 3	3.582	3.5687	7.5938	27.736	23.437	28.115	5.4	5.7452	10.649	7.6001	37.741	36.42	29.788	4.3
AT3G01450	ARM repeat superfamily protein	353.71	347.99	359.26	405.11	404.05	413.12	1.2	398.72	375.95	375.69	465.71	464	463.47	1.2
AT3G05400	Major facilitator superfamily protein	258.01	242.38	259.21	470.43	482.01	461.2	1.9	263.8	243.91	259.17	375.85	373.42	343.93	1.4
AT3G05980	unknown protein	244.78	220.87	212.84	146.84	136.62	136.04	0.6	185.68	180.14	174.5	258.11	243.83	233.73	1.4
AT3G09162	unknown protein	162.48	176.57	169.63	251.89	234.91	238.66	1.4	153.03	126.2	130.62	229.92	198.85	190.45	1.5
AT3G09405	Pectinacetyltransferase family protein	143.62	136.5	141.1	232.13	252.62	232.89	1.7	137.75	118.4	122.25	182.57	199.35	192.66	1.5
AT3G12970	unknown protein	365.78	407.21	410.92	247.19	262.72	231.67	0.6	547.02	521.98	499.74	396.47	427.75	412.32	0.8
AT3G17790	purple acid phosphatase 17	1042.9	926.25	923.43	3370.2	3551.2	3150.2	3.5	1537.6	1097.4	1453.4	3125.7	2681.6	2866.5	2.1
AT3G18200	nodulin MtN21 /EamA-like transporter family protein	275.34	286.37	249.27	154.26	160.78	144.54	0.6	240.71	249.38	269.96	346.85	348.42	325.26	1.3
AT3G19010	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	3413.7	3454.2	3647.9	4856.4	4734.5	4825.2	1.4	3998	3810.1	4069.7	4401.9	4482.8	4732.2	1.1
AT3G19550	unknown protein	51.83	57.779	47.316	215.17	213.95	170.08	3.8	247.83	152.48	212.02	350.91	345.06	326.94	1.7
AT3G22460	O-acetylserine (thiol) lyase (OAS-TL) isoform A2	1223	1148.3	1263.1	1650.4	1555	1629	1.3	1521.8	1504.5	1520	1819.7	1886.4	1843	1.2
AT3G22640	cupin family protein	95.084	84.011	100.54	31.776	46.579	30.305	0.4	428.78	618.82	459.94	205.86	252.34	217.23	0.4
AT3G22930	calmodulin-like 11	635.06	590.82	590.07	867.21	928.2	902.35	1.5	580.97	575.28	541.9	751.95	742.02	680.81	1.3
AT3G23550	MATE efflux family protein	789.59	813.99	716.39	1978.5	1605	1997.3	2.4	1083	1031.4	970.28	1840	2362.6	1862.1	2.0
AT3G24520	heat shock transcription factor C1	2002.9	2057.2	2099.4	1336.4	1306.6	1319.7	0.6	1457	1517.7	1605.8	971.05	981.04	1050.5	0.7
AT3G25240	Protein of unknown function (DUF506)	32.252	18.748	30.962	114.78	110.58	92.54	3.9	30.714	32.304	25.813	259.7	156.11	203.52	7.0
AT3G26200	cytochrome P450, family 71, subfamily B, polypeptide 22	235.64	180.67	230.12	443.21	449.42	468.12	2.1	344.16	323.83	361.99	636.84	664.51	686	1.9
AT3G27690	photosystem II light harvesting complex gene 2.3	3535.1	3178.8	3516.2	2229.9	2398.5	2262.5	0.7	5759.2	6780.7	6323.8	8720.9	8034	9547.3	1.4
AT3G28345	ABC transporter family protein	140.42	130.51	119.75	52.214	62.682	51.73	0.4	91.983	92.931	74.864	51.413	54.12	40.009	0.6
AT3G28740	Cytochrome P450 superfamily protein	1675.8	1536.3	1916	699.82	751.04	724.18	0.4	4749.9	5302	4580	3332.3	3293	3593.2	0.7
AT3G29780	ralf-like 27	375.1	351.55	359.4	214.44	250.23	196.12	0.6	212.41	211.46	215.35	333.99	334.57	321.15	1.5
AT3G44970	Cytochrome P450 superfamily protein	154.16	93.534	125.69	324.61	329.24	323.04	2.6	173.97	167.8	175.13	284.36	270.13	297.24	1.6
AT3G46280	protein kinase-related	1339.4	1653.6	1177.5	3955.6	3501.4	3547.2	2.6	1681.8	1796.8	1558	1010.2	967.82	947.71	0.6
AT3G46660	UDP-glucosyl transferase 76E12	76.415	99.866	94.665	26.774	28.172	25.556	0.3	159.38	209.19	168.76	91.474	71.568	104.71	0.5
AT3G46670	UDP-glucosyl transferase 76E11	584.8	532.27	550.73	336.21	331.96	363.17	0.6	814.61	977.31	1011.5	585.03	453.36	629.27	0.6
AT3G47420	phosphate starvation-induced gene 3	964.07	836.09	959.24	3704.5	3566.4	3516.2	3.9	1578.6	1298.2	1610.4	4427.3	3650.9	4413.7	2.8
AT3G48930	Nucleic acid-binding, OB-fold-like protein	36084	35581	34978	27287	26303	29045	0.8	28344	28851	27637	31782	31692	30827	1.1
AT3G50480	homolog of RPW8 4	23262	25297	21656	37183	34407	35439	1.5	24945	22937	23114	17293	16226	16733	0.7
AT3G53210	nodulin MtN21 /EamA-like transporter family protein	511.16	529.07	532.99	416.69	441.49	439.46	0.8	444.37	441.86	461.25	494.11	490.18	499.6	1.1
AT3G59140	multidrug resistance-associated protein 14	274.91	305.81	252.13	145.68	154.16	154.45	0.5	486.66	542.3	475.1	325.21	285.37	317.91	0.6
AT3G62270	HCO3- transporter family	873.76	888.46	901.33	699.52	691.06	636.58	0.8	897.68	800.6	859.5	620.46	690.28	597.71	0.7
AT3G62410	CP12 domain-containing protein 2	64932	70623	66092	86782	87714	86344	1.3	60273	51572	58104	76754	73344	77283	1.3
AT3G62760	Glutathione S-transferase family protein	85.254	89.845	81.009	39.11	39.183	30.719	0.4	138.13	99.576	113.96	188.97	168.12	172.68	1.5
AT3G63200	PATATIN-like protein 9	2634.9	2611.7	2555.4	1785.3	1650	1472.7	0.6	2270.2	2455.2	2248	1868.3	2013.4	1763.1	0.8
AT4G02330	Plant invertase/pectin methylesterase inhibitor superfamily	460.39	440.06	430.15	1097.9	1017.4	1012.6	2.4	567.36	567.59	557.06	344.08	341.37	315.04	0.6
AT4G03510	RING membrane-anchor 1	253.7	253.87	229.6	464.88	432.53	428.7	1.8	332.91	416.75	311.88	618.84	591.57	557.63	1.7
AT4G03960	Phosphotyrosine protein phosphatases superfamily protein	308.77	317.57	356.19	680.82	695.28	610.89	2.0	299.36	288.24	333.39	917.23	727.11	896.85	2.8
AT4G12470	azelaic acid induced 1	44929	58649	48649	89776	83515	87714	1.7	48852	47648	47326	30306	28144	32251	0.6
AT4G14030	selenium-binding protein 1	4373.4	4367.3	3938.8	5909.1	5784.1	5906.5	1.4	4438.7	4929.6	4348.7	5962.7	6221.5	5653.7	1.3
AT4G15540	EamA-like transporter family	1009.6	981.44	1099.7	1708.6	1720.7	1808.9	1.7	1291.3	1186.9	1357.1	2116.9	2188	2275.1	1.7
AT4G21870	HSP20-like chaperones superfamily protein	4441	4757.1	5036.5	7284.4	7011	7862.9	1.6	5475.4	5605.2	5261.4	8649.1	7627.8	8261.4	1.5
AT4G23130	cysteine-rich RLK (RECEPTOR-like protein kinase) 5	246.53	273.99	236.16	452.05	418.73	401.14	1.7	165.39	172.11	162.64	206.75	204.08	189.2	1.2
AT4G25630	fibrillarlin 2	13522	14662	14799	9823.1	9729.7	10778	0.7	13238	13190	13622	8712.6	7768.2	9326.3	0.6
AT4G26260	myo-inositol oxygenase 4	683.84	609.45	622.33	1611.1	1736.7	1578.1	2.6	581.61	602.71	528.64	1375.1	1512.2	1303.1	2.4

AT4G31350	Core-2/1-branching beta-1,6-N-acetylglucosaminyltransferase family protein	1506.9	1425	1506.5	1742.9	1778.7	1807.9	1.2	1350.9	1305.2	1379.3	1574.7	1556.3	1640.2	1.2
AT4G32785	unknown protein	82.265	84.992	84.679	54.416	62.124	54.093	0.7	141.68	125.56	125.67	95.719	86.031	103.41	0.7
AT4G33420	Peroxidase superfamily protein	772.83	708.4	666.08	1118.2	1027.6	1055.8	1.5	743.92	682.26	767.02	612.7	562.27	596.56	0.8
AT4G33985	Protein of unknown function (DUF1685)	1176.7	1182.3	1129.6	1626.7	1550.9	1508.1	1.3	1403.9	1295.8	1348.2	1675	1667	1652.9	1.2
AT4G34135	UDP-glucosyltransferase 73B2	763.73	694.24	710.92	496.32	480.42	540.25	0.7	1330.2	1633.1	1569.2	846.45	697.47	868.15	0.5
AT4G34419	unknown protein	282.54	262.06	278.08	189.61	172.66	191.26	0.7	247.14	223.04	226.34	370.12	334.4	333.12	1.5
AT4G34980	subtilisin-like serine protease 2	3422.7	3489.7	3243.8	2717.8	2789.3	2732.1	0.8	4133.8	4286.8	4129.1	3545.3	3710.7	3654.3	0.9
AT4G36820	Protein of unknown function (DUF607)	179.91	142.58	176.38	292.21	285.6	321.1	1.8	170.59	178.4	170.06	295.86	325.36	302.61	1.8
AT4G37880	LisH/CRA/RING-U-box domains-containing protein	2496.6	2552.7	2535.4	2783.2	2728.1	2759.7	1.1	2506.8	2508.7	2549.4	3096.9	3042.6	2864	1.2
AT4G39770	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein	138.08	153.44	139.91	83.35	78.027	93.534	0.6	125.98	128.15	138.15	100.58	107.24	106.84	0.8
AT4G39795	Protein of unknown function (DUF581)	534.63	532.14	563.07	281.63	293.73	242.01	0.5	253.5	239.77	216.75	422.43	475.18	427.75	1.9
AT5G02270	non-intrinsic ABC protein 9	972.4	909.11	962.88	720.54	745.09	679.73	0.8	757.57	757.27	726.82	890.72	856.03	866.55	1.2
AT5G02580	Plant protein 1589 of unknown function SAUR-like auxin-responsive protein family	296.11	290.19	314.59	213.19	199.44	189.39	0.7	225.25	237.43	260.74	397.32	389.29	394.2	1.6
AT5G03310	unknown protein	217.06	207.81	232.45	340.94	335.87	332.05	1.5	197.92	187.07	191.38	337.59	382.57	312.96	1.8
AT5G05300	unknown protein	24.418	16.107	28.467	74.973	74.139	67.846	3.1	38.421	39.26	39.377	55.024	58.292	58.482	1.5
AT5G05440	Polyketide cyclase/dehydrase and lipid transport superfamily protein	2878	2755.7	3162.4	4272.9	4135.2	4454	1.5	2953.5	2671.9	2529.1	3943.9	3882.5	4089.5	1.5
AT5G10695	unknown protein	599.32	579.46	611.91	834.7	800.17	810.29	1.4	660.76	623.24	630.89	761.67	871.67	807.99	1.3
AT5G14020	Endosomal targeting BRO1-like domain-containing protein	253.72	263.01	237.42	126.76	153.44	122.11	0.5	194.74	182.24	181.29	233.34	233.08	224.77	1.2
AT5G14570	high affinity nitrate transporter 2.7	407.21	434.47	424.4	317.66	341.64	315.65	0.8	625.05	639.76	601.88	499.74	521.62	486.77	0.8
AT5G14640	shaggy-like kinase 13	434.36	423.19	440.52	345.89	371.55	351.55	0.8	591.32	543.67	585.41	380.83	369.5	387.74	0.7
AT5G20150	SPX domain gene 1	3783.7	3785.9	3790.9	8852.9	10274	8692.2	2.4	5113	4756.2	4811.9	16930	15154	17503	3.4
AT5G20790	unknown protein	146.7	127.18	142.22	640.43	581.99	543.99	4.2	153.96	161.15	144.88	1176.6	630.03	1072.8	6.3
AT5G23070	Thymidine kinase	242.06	235.17	223.44	182.97	181.45	179.82	0.8	267.27	309.9	282.93	219.48	213.21	206.79	0.7
AT5G24160	squalene monooxygenase 6	171.79	180.95	149.33	368.24	361.02	352.4	2.2	447.4	449.7	469.14	335.53	375.51	327.16	0.8
AT5G24640	unknown protein	55.896	87.188	64.279	291.78	336.6	278.41	4.4	874.1	899.72	817.26	1115.4	1101.9	1131.3	1.3
AT5G36130	Cytochrome P450 superfamily protein	149.06	118.52	135.87	17.456	17.563	28.263	0.2	30.64	34.398	31.449	22.12	20.491	25.175	0.7
AT5G36925	unknown protein	128.14	135.61	128.89	519.03	501.18	461.69	3.8	138.05	120.7	120.15	177.11	201.59	178.44	1.5
AT5G41761	unknown protein	2016.7	1386.5	2021.3	3759.5	3697.5	3635	2.0	1463.7	1253.7	1223.6	2568.2	2401.6	2291.7	1.8
AT5G43300	PLC-like phosphodiesterases superfamily protein	76.242	81.966	71.719	114.17	110.87	114.11	1.5	59.216	73.494	60.102	106.47	108.67	123.61	1.8
AT5G44578	unknown protein	62.901	58.994	58.788	205.41	158.49	170.5	3.0	46.725	65.925	51.588	94.535	86.441	91.474	1.7
AT5G46871	Putative membrane lipoprotein	61.876	81.636	84.11	258.67	257.79	257.79	3.4	238.16	218.24	273.82	510.3	567.98	567.82	2.3
AT5G48940	Leucine-rich repeat transmembrane protein kinase family protein	122.36	142.14	118.25	59.887	59.464	58.72	0.5	182.61	188.14	186.58	133.62	123.83	128.63	0.7
AT5G52520	Class II aaRS and biotin synthetases superfamily protein	1589.9	1668.7	1703.6	1320	1307.1	1366.2	0.8	2181	2399.4	2428.5	1641.6	1587.8	1919.9	0.7
AT5G52790	CBS domain-containing protein with a domain of unknown function (DUF21)	62.44	68.448	60.959	38.647	37.503	34.542	0.6	78.935	72.411	71.878	56.304	46.925	52.927	0.7
AT5G52960	unknown protein	10533	10559	10737	9882.3	9715.5	9708.6	0.9	9855.2	10017	9182.9	12435	12626	12833	1.3
AT5G56120	unknown protein	1153	1173.9	1141	920.6	869.36	922.22	0.8	1025.4	1088	1023.1	1330.2	1322.8	1324.1	1.3
AT5G57870	MIF4G domain-containing protein / MA3 domain-containing protein	16634	16569	16850	15218	15019	15238	0.9	16129	15700	15247	13509	14342	13946	0.9
AT5G60890	myb domain protein 34	1297	1350.7	1251.3	851.77	864.11	821.68	0.7	1048.4	1041.1	1094.4	853.34	875.72	791.72	0.8
AT5G61020	evolutionarily conserved C-terminal region 3	2188.4	2153.4	2143.6	1819.4	1911.1	1887.7	0.9	2659.7	2686.6	2485.9	1783.1	1900	1779.7	0.7
AT5G62440	Protein of unknown function (DUF3223)	1085.1	1079.8	1135.7	889.76	814.24	848.93	0.8	1198.1	1290.1	1328.5	760.73	710.36	721.71	0.6
AT5G63580	flavonol synthase 2	87.244	68.144	77.841	152.81	139.27	141.39	1.9	85.899	90.411	87.971	36.78	47.231	57.592	0.5
AT5G63850	amino acid permease 4	741.71	924.08	691.31	2232.7	2102.4	1893.8	2.6	2595.8	2340.2	2203.5	1692.6	1793.9	1549.8	0.7
AT5G67230	Nucleotide-diphospho-sugar transferases superfamily protein	311.8	269.24	294.68	403.89	398.49	390.39	1.4	316.76	333.51	309.08	435.84	456.5	434.41	1.4
AT5G67500	voltage dependent anion channel 2	1638.3	1589.9	1590.4	1417.3	1407.3	1364.9	0.9	1997.7	1898.8	2012.3	1234	1325.3	1373.6	0.7
AY536062	Full-length cDNA	8192.1	7099.8	8046.3	17308	17000	16358	2.2	8565.7	7504	7842.5	21783	17302	21443	2.5
BX817525	Full-length cDNA	1647.9	1449.5	1629.5	1026.5	1074.3	992.78	0.7	968.31	1001.8	898.32	1227.7	1194.4	1128.7	1.2
BX828992	Full-length cDNA	642.97	682.13	642.21	950.21	910.13	881.76	1.4	605.27	490.68	487.19	773.69	724.85	769.48	1.4
DQ108818	Full-length cDNA	53.474	38.661	48.566	124.71	125.42	110.54	2.6	54.844	59.481	48.837	82.892	87.676	87.927	1.6
EF183020	Full-length cDNA	55.918	53.127	67.949	133.83	131.5	155.86	2.4	113.99	92.386	118.63	220.32	225.53	294.51	2.3
EF183376	Full-length cDNA	731.28	772.15	760.76	638.59	648.33	657.35	0.9	665.92	760.04	662.87	898.32	888.94	869.85	1.3
RAFL06-10-F1	Full-length cDNA	1486.7	1486.4	1575.2	2091.4	2175.1	2007.4	1.4	1406.5	1342.2	1277	1830.7	1849	1692.4	1.3
RAFL07-16-D	Full-length cDNA	2422	2335.1	2448.2	2912.8	2874.5	2868.7	1.2	1927	2137.8	1961.3	1421.5	1393.5	1494.8	0.7
RAFL09-47-E1	Full-length cDNA	156.61	159.12	150.75	81.131	92.644	85.328	0.6	155.17	158.81	154.36	131.32	130.74	135.46	0.8
RAFL17-06-E1	Full-length cDNA	286.06	271.85	290.42	416.95	393.11	440.64	1.5	312.56	330.55	328.83	456.5	446.86	490.4	1.4
RAFL19-20-E1	Full-length cDNA	933.27	1011.5	988.91	1382.3	1383.7	1263.1	1.4	1091.9	1071.2	1000.7	1558.2	1426.1	1394.9	1.4

RAFL19-64-O	Full-length cDNA	175.21	178.76	184.64	237.42	237.29	223.53	1.3	185.23	185.31	156.53	266.93	283.66	259.28	1.5
RAFL19-65-G	Full-length cDNA	900.84	836.26	840.24	1300.4	1369	1210.9	1.5	930.52	951.07	838.83	1376.2	1361.6	1197.2	1.4
RAFL24-01-H	Full-length cDNA	153.12	152.99	156.38	125.78	121.85	118.25	0.8	139.05	154.19	163.07	181.27	194.93	193.68	1.2
RAFL25-22-I1	Full-length cDNA	551.24	429.57	585.6	2413.6	2571.1	2374.7	4.7	707.14	561.23	679.12	2069.5	1864.2	2297.9	3.2
sORF2655	chr2_+_6564522-6564674	1694.6	1738.5	1710.1	1325.8	1342.7	1371.1	0.8	1021.6	1069.4	1070.3	1547.3	1337	1343.5	1.3
sORF4533	chr3_-15205819-15205971	1183.4	1279.1	1224.2	911.49	932.11	921.73	0.8	685.08	706.15	711.08	1021.3	912.93	995.19	1.4
sORF0154	chr1_-2407651-2407785	167.55	142.44	122.66	385.49	380.72	372.66	2.6	187.7	163.41	132.36	576.19	460.21	405.14	3.0

a) AGI: Arabidopsis gene ID and gene description from TAIR ver. 10

b). Full length cDNA IDs (<http://www.brc.riken.jp/lab/epd/catalog/cdnaclone.html> or <http://www.ncbi.nlm.nih.gov/genbank/>)

b) Small open reading frames (sORFs) identified in our previous paper (Hanada et al., PNAS 2013)

Table S5. Gene ontology analysis of up- and down-regulated genes in *AtPROPEP3/AT13-ox* plants and *AtPep3/AT13* peptide treated plants

GO category ^{a)}	Gene names	Gene count	Annotation count	FDR
positive_regulation_of_cellular_response_to_phosphate_starvation:GO:0080040	AT2G45130,AT5G20150	2	3	3.76E-27
phosphate_ion_transport:GO:0006817	AT1G68740,AT1G26730,AT2G45130,AT5G20150	4	29	1.04E-12
galactolipid_biosynthetic_process:GO:0019375	AT1G68740,AT1G67600,AT1G08310,AT1G17710,AT2G45130,AT3G17790,AT5G20150	7	99	1.34E-07
anaerobic_respiration:GO:0009061	AT2G41730,AT5G23070	2	20	3.09E-05
syncytium_formation:GO:0006949	AT2G39700,AT4G26260	2	21	4.75E-05
cellular_response_to_phosphate_starvation:GO:0016036	AT1G68740,AT1G67600,AT1G26730,AT1G08310,AT1G17710,AT2G45130,AT3G17790,AT5G20150	8	168	1.80E-04
negative_regulation_of_transcription_DNA-templated:GO:0045892	AT1G68740,AT1G67600,AT1G08310,AT1G17710,AT2G45130,AT3G17790,AT5G20150	7	165	1.44E-03
defense_response_to_insect:GO:0002213	AT3G28740,AT5G60890	2	34	4.65E-03
fatty_acid_catabolic_process:GO:0009062	AT3G46660,AT3G53210,AT5G02580	3	62	4.65E-03
plant-type_cell_wall_loosening:GO:0009828	AT2G20750,AT2G39700	2	35	5.10E-03
flavonoid_biosynthetic_process:GO:0009813	AT3G19010,AT5G63580,AT5G02270	3	65	5.40E-03
para-aminobenzoic_acid_metabolic_process:GO:0046482	AT1G05560,AT2G29490	2	36	5.40E-03
positive_regulation_of_flavonoid_biosynthetic_process:GO:0009963	AT2G18730,AT2G21560,AT2G28660,AT3G19010	4	103	9.85E-03
amino_acid_transport:GO:0006865	AT1G80450,AT1G10585,AT3G46280,AT4G12470,AT5G63850	5	144	1.48E-02
anion_transport:GO:0006820	AT3G62270,AT5G67500	2	46	2.41E-02

a) Genes were functionally categorized using GO slim in TAIR.

Table S6. No significant change of gene expression related to other hormones

Gene	Related Hormone	Function category	Symbol	AT13ox/ WT	AT13pep/ DMSO
AT2G40940	ethylene	Hormone receptor	ERS1	0.86	1.27
AT3G24520	abscisic acid	Hormone signal transduction	HSFC1	0.66	0.64
AT4G12470	salicylic acid	Hormone signal transduction	AZI1	0.63	1.71
AT5G03310	auxin	Hormone signal transduction	SAUR44	1.79	1.53
AT5G05440	abscisic acid	Hormone receptor	PYL5	1.46	1.46
AT5G67500	abscisic acid	Hormone signal transduction	VDAC2	0.67	0.87