***Populus euphratica* XTH overexpression enhances salinity tolerance by the development of leaf succulence in transgenic tobacco plants**

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**Supplementary Table S1.** Accession numbers of XTH protein sequences used in multiple sequence alignment and phylogenetic analysis.

| Sequence name | Accession number | Sequence name | Accession number |
|---|---|---|---|
| AtXTH1 | NP_193044 | AtXTH2 | NP_193045 |
| AtXTH3 | NP_189141 | AtXTH4 | NP_178708 |
| AtXTH5 | NP_196891 | AtXTH6 | NP_569019 |
| AtXTH7 | NP_195494 | AtXTH8 | NP_563892 |
| AtXTH9 | NP_192230 | AtXTH12 | NP_200561 |
| AtXTH13 | NP_200562 | AtXTH14 | NP_194312 |
| AtXTH15 | NP_193149 | AtXTH16 | NP_566738 |
| AtXTH17 | NP_176710 | AtXTH18 | NP_194757 |
| AtXTH19 | NP_194758 | AtXTH20 | NP_199618 |
| AtXTH21 | NP_179470 | AtXTH24 | NP_194756 |
| AtXTH27 | NP_178294 | AtXTH30 | NP_174496 |
| AtXTH31 | NP_190085 | AtXTH32 | NP_181224 |
| CsXTH1 | CAD87535 | FpXET1 | CAC40807 |
| FpXET2 | CAC40808 | FsXTH | CAA10231 |
| HvXEA | P93671 | HvXEB | P93672 |
| NtEXT1 | BAA32518 | NtEXT2 | BAA13163 |
| NtXTH | ADV41673 | PtXTH | XP_002318936 |
| PtXTH1 | ABK92523 | PtXTH2 | ABK95902 |
| PtXTH3 | ABK94576 | PtXTH4 | ABK92940 |
| PtXTH5 | ABK95370 | PttXTH16-1 | ABM91065 |
| PttXTH16-3 | ABM91070 | PttXTH16-14 | ABM91071 |
| PttXTH16-17 | ABM91073 | PttXTH16-21 | ABM91075 |
| PttXTH16-26 | ABM91063 | PttXTH16-27 | ABM91069 |
| PttXTH16-30 | ABM91074 | PttXTH16-34 | AAN87142 |
| PttXTH16-35 | ABL75361 | PttXTH16-36 | ABM91067 |
| PttXTH16-39 | ABM91072 | RcXTH | XP_002526228 |
| SlXTH1 | BAA03923 | SlXTH4 | AAG43444 |
| SlXTH5 | AAS46240 | SlXTH6 | AAS46242 |
| SlXTH8 | BAA88668 | SlXTH12 | AAF17600 |
| SpXTH1 | BAE06063 | TaXTH1 | AAT94293 |
| TaXTH3 | AAT94295 | TaXTH4 | AAT94296 |
| TaXTH5 | AAT94297 | VaXTH1 | Q41638 |
| VaXTH2 | BAC03238 | VvXTH | XP_002267890 |
| ZmXTH1 | NP_001105166 | ZmXTH2 | ACF85167 |
| ZmXTH3 | NP_001151500 | ZmXTH4 | NP_001130486 |
| ZmXTH5 | ADB54615 | ZmXTH6 | ACF88385 |

Protein sequences were obtained from NCBI RefSeq, GenBank database, and UniProtKB/Swiss-Prot database.

**Supplementary Table S2.** Primers used for quantitative real-time PCR.

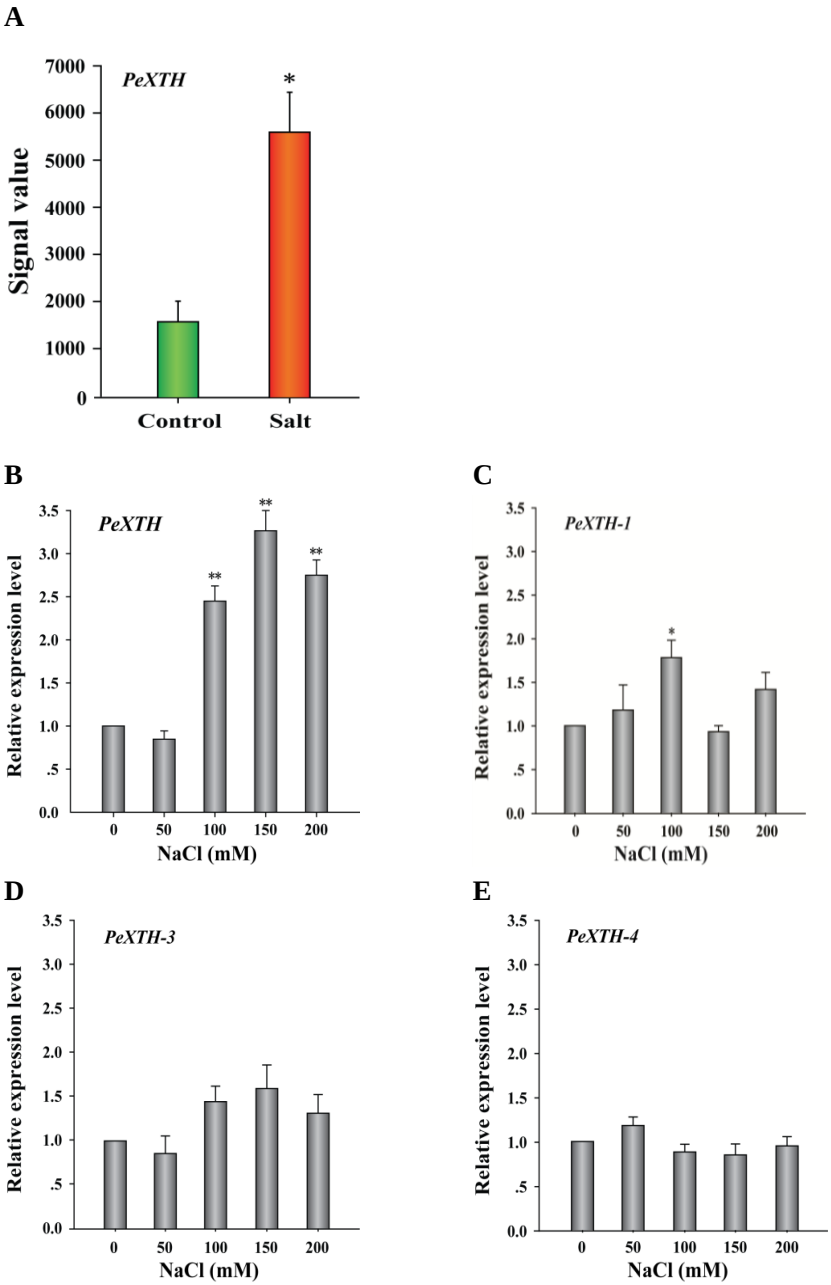
| Target | Primer name | Primer sequence (5’ to 3’) |
|---|---|---|
| *PeXTH* (in *P. euphratica*) | Forward primer | AAAGGGTCTGCGTGGGATG |
| | Reverse primer | CGGGAGGAGAAGTGGTTG |
| *PeXTH* (in tobacco) | Forward primer | TTAGCCAAGGCAAAGGCAAC |
| | Reverse primer | AGCCCAGTCATCAGCATTCC |
| *PeACT7* | Forward primer | ATGCTGCTAGGAGCCAGTGC |
| | Reverse primer | TTGTGCTCAGTGGTGGCTCTAC |
| *NtEF1α* | Forward primer | GCTGTGAGGGACATGCGTCAAA |
| | Reverse primer | GTAGTAGATATCGCGAGTACCACCA |
| *NtXTH* (D86730) | Forward primer | GCTAGTCACCACATCAAGTA |
| | Reverse primer | CTGAGTCTCCACCAACAAG |
| *NtXTH* (AB017025) | Forward primer | CAGAGCACGATGAGATAGAT |
| | Reverse primer | TGATGTGTAGGAGGCAGTA |
| *PeXTH-1* | Forward primer | GTGATTCTGCTGGAACTGT |
| | Reverse primer | GGTTCTCCTGTGGTGTTG |
| *PeXTH-3* | Forward primer | TCCACTACTATTCTGTCCTCT |
| | Reverse primer | CCAATCATCGGCATTCCA |
| *PeXTH-4* | Forward primer | CAGAGCACGATGAGATAGAT |
| | Reverse primer | TGATGTGTAGGAGGCAGTA |

In this study the PCR conditions were: 95℃ for 5 min, followed by 34 cycles of 94℃ for 30 s, 55℃ for 30 s, and 72℃ for 30s, with a final step of 72℃ for 10 min.

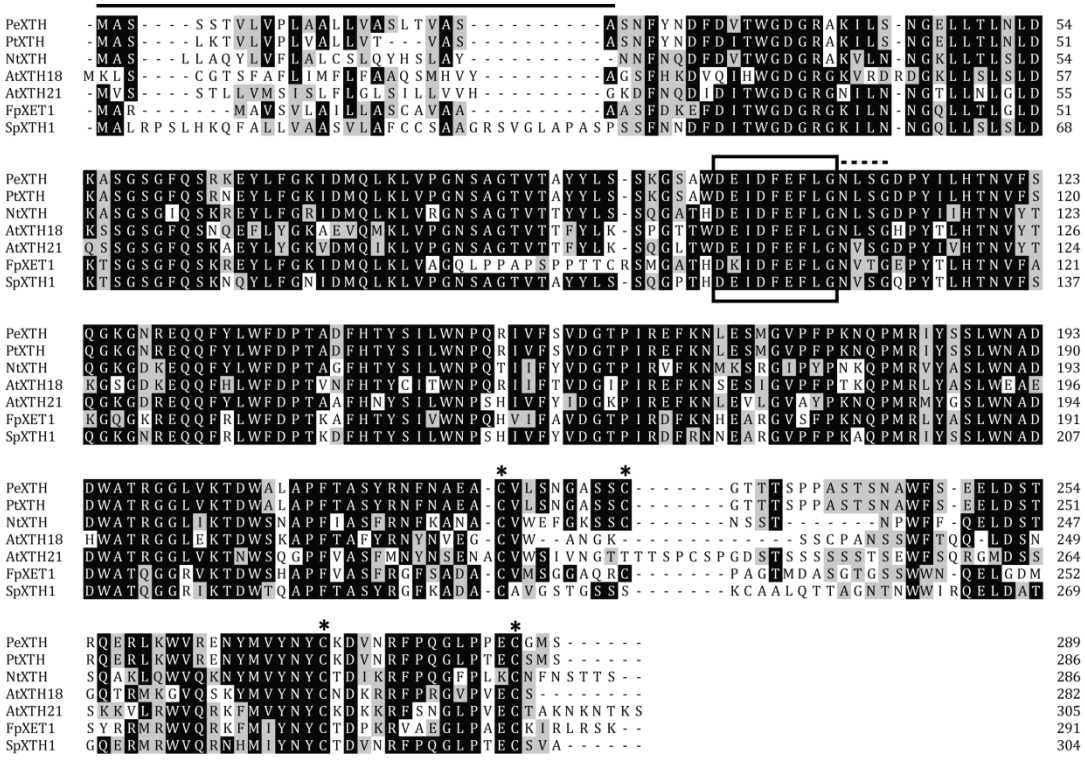
**Supplementary Table S3.** The segregation ratio of kanamycin-resistant ($Kan^R$) to kanamycin-sensitive ($Kan^S$) seedlings among $T_1$ progeny of *PeXTH*-transgenic plants.

| Line | $Kan^R/Kan^S$ | Segregation ratio ($\chi^2_{0.05}$ test ) |
|---|---|---|
| WT | 0/107 | 0 |
| L5 | 78/23 | $3.4:1^{N.S.}$ |
| L6 | 98/25 | $3.9:1^{N.S.}$ |
| L8 | 82/30 | $2.7:1^{N.S.}$ |
| L14 | 89/25 | $3.6:1^{N.S.}$ |

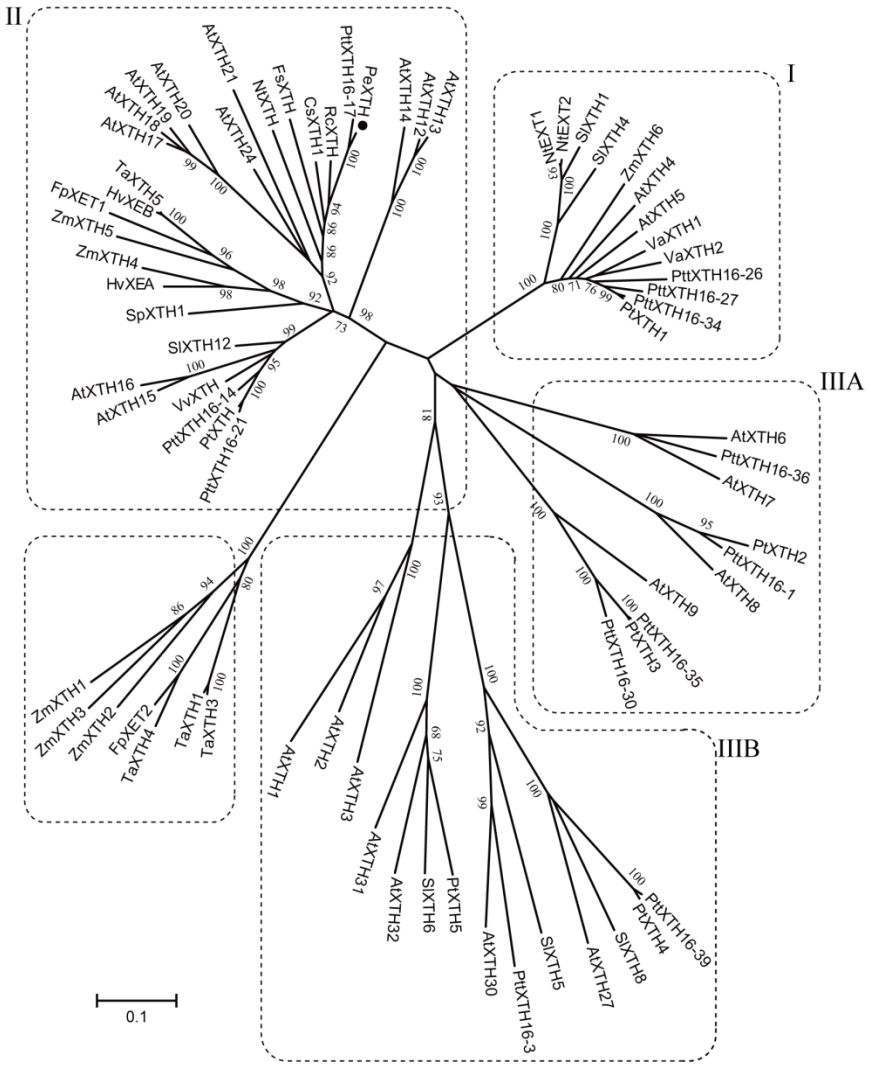
N.S. indicates no significant difference between the transgene segregation ratio and the expected 3:1 Mendelian segregation for a single insertion site ($P > 0.05$).



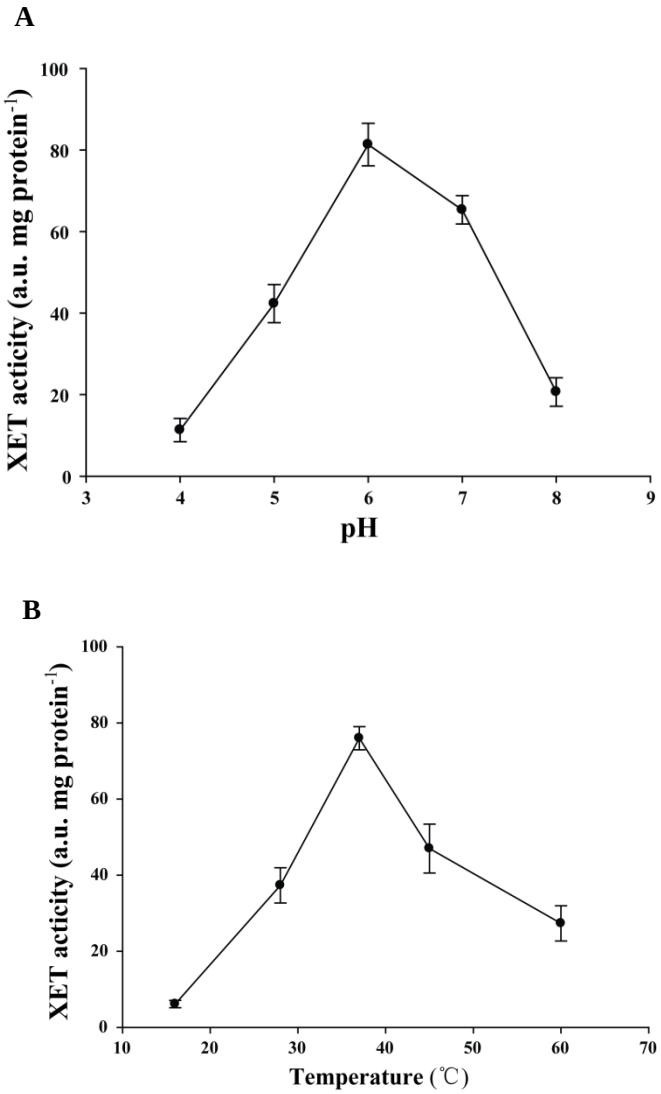

**Supplementary Fig. S1.** Expression profiles of *XTH* isoforms in *P. euphratica* leaves under salt stress. (A) Microarray analysis of *PeXTH* using the Affymetrix Poplar Array (Ding *et al.*, 2010; Affymetrix; Santa Clara, CA, USA). *PeXTH* gene (Probeset ID: PtpAffx.120153.1.S1-S-at; an *Arabidopsis thaliana* ortholog) was up-regulated in *P. euphratica* after exposure to 200 mM NaCl for 28 days. (B-E) Real-time quantitative PCR analysis of four *XTH* isoforms (*PeXTH*, *PeXTH-1*, *PeXTH-3*, and *PeXTH-4*). *P. euphratica* seedlings were subjected to 4 weeks of increasing NaCl, which started from 50 mM and increased stepwise by weekly 50 mM, reaching 200 mM NaCl in the fourth week. Total RNA was extracted from leaves of *P. euphratica*. Primers designed to target these *XTH* genes (Supplementary Table S2) were based on *P. trichocarpa* homologs obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/). *ACT7* was used as an internal control. Each column is the mean of three individual plants and bars represent the standard error of the mean. * $P < 0.05$, ** $P < 0.01$ control vs. salt treatment.



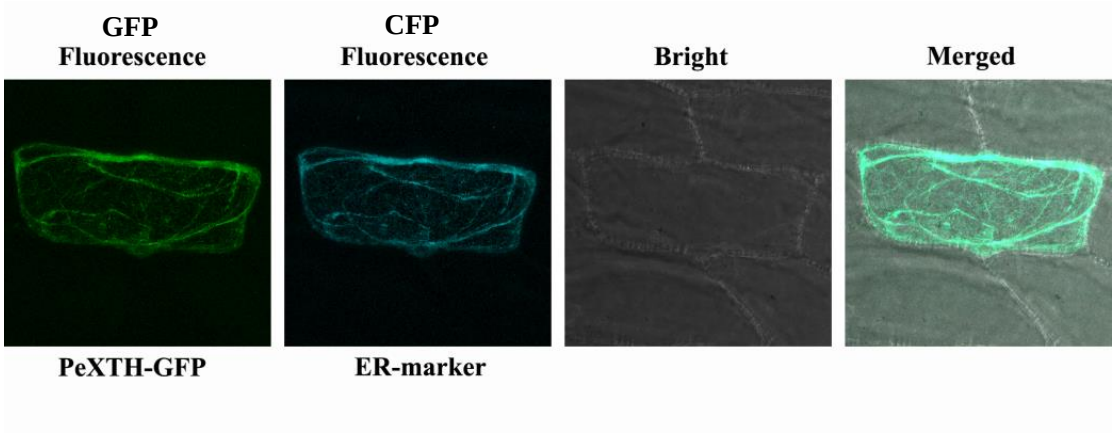

**Supplementary Fig. S2.** Multiple sequence alignment of PeXTH (xyloglucan endotransglucosylase/hydrolase from *P. euphratica*) with other XTHs from different species. The XTH sequences are from *Arabidopsis thaliana* (AtXTH18, AtXTH21), *Festuca pratensis (FpXET1), Nicotiana tabacum (NtXTH), Populus euphratica (PeXTH), Populus trichocarpa (PtXTH),* and *Sagittaria pygmaea (SpXTH1).* The accession numbers of these sequences are provided in Supplementary Table S1. Black and grey shading indicate identical and conserved amino acid residues, respectively. Residues enclosed in a box indicate the conserved DEIDFEFLG catalytic domain of XTHs. The putative N-glycosylation motif immediately following the catalytic domain is marked by a dotted line. The four conserved Cys residues (C) are labeled by asterisk (*). The putative signal peptide sequence of PeXTH is marked by a solid line.



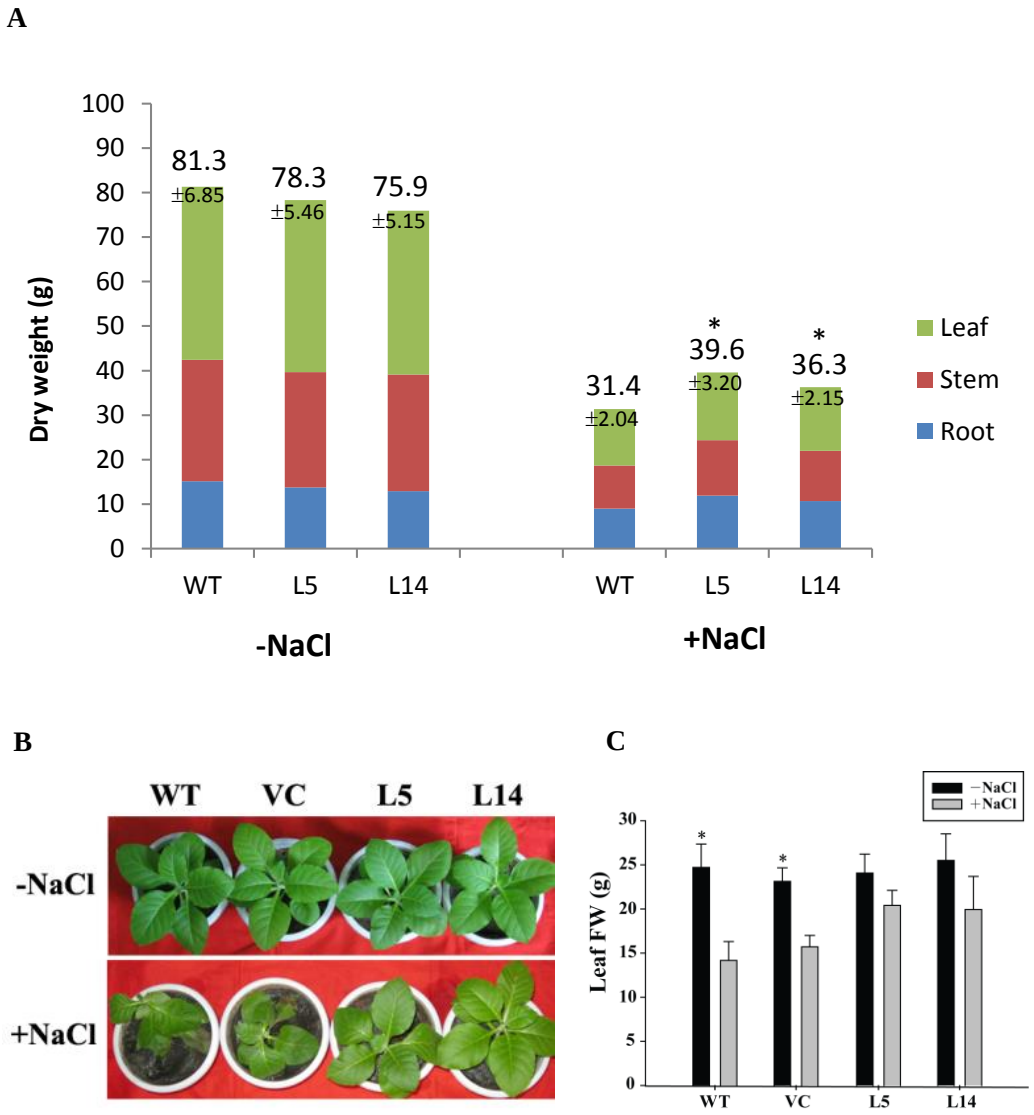

**Supplementary Fig. S3.** Phylogenetic relationships between PeXTH and other representative XTH proteins from different plant species. Seventy-three amino acid sequences of XTH (without signal peptides) were used for phylogenetic analysis. The numbers at the branches indicate bootstrap percentages (values < 70% are not shown). The different species are indicated as follows: At, *Arabidopsis thaliana*; Cs, *Cucumis sativus* (cucumber); Fp, *Festuca pratensis*; Fs, *Fagus sylvatica* (European beech); Hv, *Hordeum vulgare* (barley); Nt, *Nicotiana tabacum* (tobacco); Os, *Oryza sativa* (rice); Pe, *Populus euphratica*; Pt, *Populus trichocarpa*; Ptt, hybrid aspen *Populus tremula* L. × *P. tremuloides* Michx; Rc, *Ricinus communis* (castor); Sl, *Solanum lycopersicum* (tomato); Sp, *Sagittaria pygmaea*; Ta, *Triticum aestivum* (wheat); Va, *Vigna angularis* (azuki bean); Vv, *Vitis vinifera* (grape); Zm, *Zea mays* (maize). The accession numbers of the XTH sequences are provided in Supplementary Table S1.



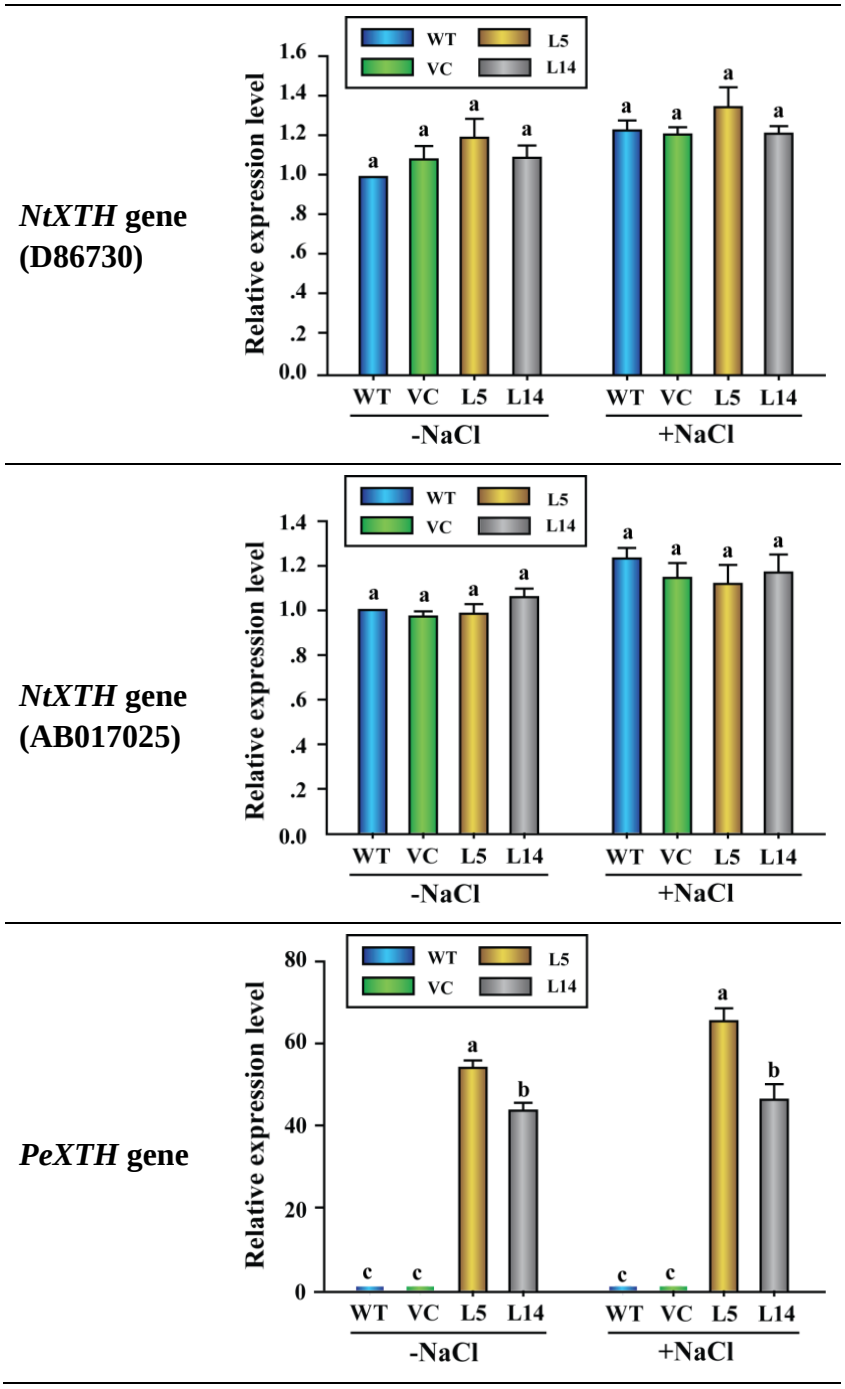

**Supplementary Fig. S4.** Effects of pH (A) and temperature (B) on xyloglucan endotransglucosylase (XET) activity of purified PeXTH protein. PeXTH protein was recombinantly produced using the prokaryotic expression system (*Escherichia coli* BL21) and purified with Ni-Sepharose media (GE Healthcare, USA). XET activity of purified PeXTH protein was tested at various pH values (4.0-8.0) and temperature (16-60$^{o}$C). Each point is the mean of three independent replicates. Bars represent the standard error (SE) of the mean.




**Supplementary Fig. S5.** Co-localization of PeXTH–GFP with the endoplasmic reticulum marker (ER-ck *CD3-953*) in non-plasmolysed onion cells. Onion epidermal cells were bombarded with 5 μg of the recombinant plasmids (*PeXTH–GFP*) with the endoplasmic reticulum marker (cyan fluorescent protein (CFP), ER-ck *CD3-953*), using a biolistic PDS-1000/He particle delivery system (Bio-Rad, Hercules, CA, USA). GFP and CFP fluorescence was measured with Leica SP5 confocal microscope (Leica Microsystems GmbH, Wetzlar, Germany). The confocal settings were excitation at 488 nm (GFP) and 453 nm (CFP), and emission at wavelength of 510-535 nm (GFP) and 460-485 nm (CFP), respectively.




**Supplemental Fig. S6.** Salt tolerance of wild-type tobacco and *PeXTH*-transgenic plants grown in hydroponics and nursery soil supplemented or not with NaCl. (A) Long-term of hydroponic culture. Wild-type (WT) tobacco and *PeXTH*-overexpressing lines (L5 and L14) were subjected to 80 days of increasing salt stress. NaCl saline increased weekly from 50 to 200 mM, and then kept at 200 mM until the end of experiment. Dry weights of root, leaf, and stem are shown. Each column labeled with values (±SE) is the mean of three plants. * $P < 0.05$, WT vs. *PeXTH*-transgenic lines. (B-C) Soil culture. In this series, wild-type, vector control (VC), and the two transgenic lines (L5 and L14) germinated on half-strength MS medium, were grown in nursery soil for 30 days. Salt treatment was applied by top watering of 150 mM NaCl. Wilted leaves were only seen in WT and VC after 7 days of salt treatment (B). Leaves were harvested from control and salt-stressed plants and fresh weight (FW) were obtained (C). Each column is the mean of three tobacco plants. The bars indicate the standard error of the mean. * $P < 0.05$ control vs. salt treatment.




**Supplementary Fig. S7.** Expression of *PeXTH* gene and two tobacco-intrinsic *NtXTH* genes in wild-type (WT), vector control (VC) and *PeXTH*-transgenic tobacco plants (L5 and L14). Accession numbers of the two *NtXTH* genes are D86730 and AB017025 (Supplementary Table S2). Plants were acclimated to one-quarter-strength Hoagland nutrient solution for 7 days and then treated with 0 and 150 mM NaCl for additional 7 days. The expression levels were detected by real-time PCR analysis using the primers listed in Supplementary Table S2. *EF1α* was used as the internal control. The bars represent the standard error of the mean. Columns labeled with different letters indicate significant differences among the wild-type, vector control and transgenic lines at $P < 0.05$ under control (-NaCl) and salt treatment (+NaCl).