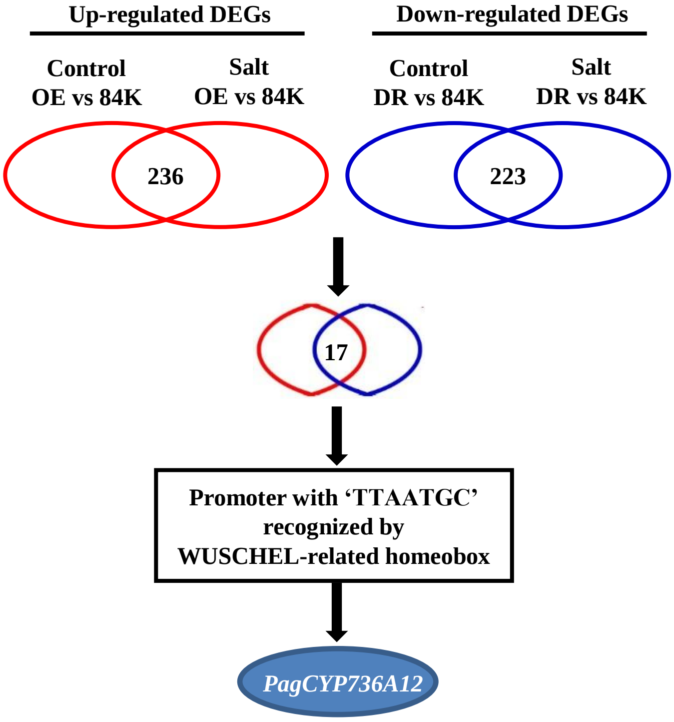

Figure S1 Screening *PagCYP736A12* gene from transcriptome data.

>Pro$_{\mathbf{PagCYP736A12}}$

AGCATCTAATGTTACGAATGGTGTTGAGACTTGTTAGGGCGACACAACTTGATTTTTCC

CCTAAATGGCTACACCGTTTGAATTAAGAGTTGTACTTGATTAATAAGTTTTTGGAATTC
TGGTTGGTAGAAGTACCTGCATTAACAGGTAGATGTACTACGCAAGTTGTAAAAAGAA

WOX-binding motif

AACTAATGGTCTTAAAAGGTGAAGCACAAGTTGTAAAAAGAAAAGTCTTTTCAAAGTG

CTTGGGAGTGTGGTTGTTGTTGTTTTTCAAAGTGTTTTTCACTTAGAAAAACATGCCAA
TAATATTTTTTTTTTATTTTTTAAAAATTATTTTTGAGATTAGCACATCAAAATGACTTGA
AAACATCAAAAGCATATTTAAAGCTAAAAACAATAAAAAAAAATTCAAATTTTTTCAG
AAGTGCTTTTGTAAAGCACTCCCAAACAAGAAATGTGAAGCACAAGTTGTAAAAAGA
AAAGTCTTAAAACGTGAAGCACAAGTTGTAAAAAAAAAAGTCTTAAAACGTGAAGCA
CAAGTGTGGTTTGGCTTTGGCTAGATGATAAAAGCTCACAAAATTATGTGGTTTGGCTT
CTCACTAAATTATGTGGTTTGGCTTTGGCTAGATAATAAAATCTCACTAAATTATGTGGT
TTGGCTTTGGCTTTCCCAAACATGACTAACTGTTCATCCGGCGGAAGGTTTTCATATCC
CAAGTGTCTATGTCCCACTCTTACACGCTAGAAACCACACCTTTATACTCACATTGATGT
CTTTTTCTTGATTGATTTCCTCCTACTAGTGGAATTAGTGGAACCACGTTGAGAGAAAT
AATTGAGTTCACACATATTCACAATTGATTGATCACTTTGTTTTCTTCCTGTTACCATCTC
CCTAGTTACTGCTATGTGCAGCGTCTTCTCTATATGAAGCAAGAGTTGGATTTAGGTTTG
CTTTCACTAAGATAGAGCATAGCGCCGAAACTTAATAAATCAGACATAGC**ATG**

Figure S2 Promoter sequence of the *PagCYP736A12* gene. The WOX11-binding motif is shown in green color and the PCR primers for the amplification of promoter fragment used in Y1H, transient expression, and ChIP assays are indicated by a solid line. The start codon (ATG) is labeled with a rectangle.

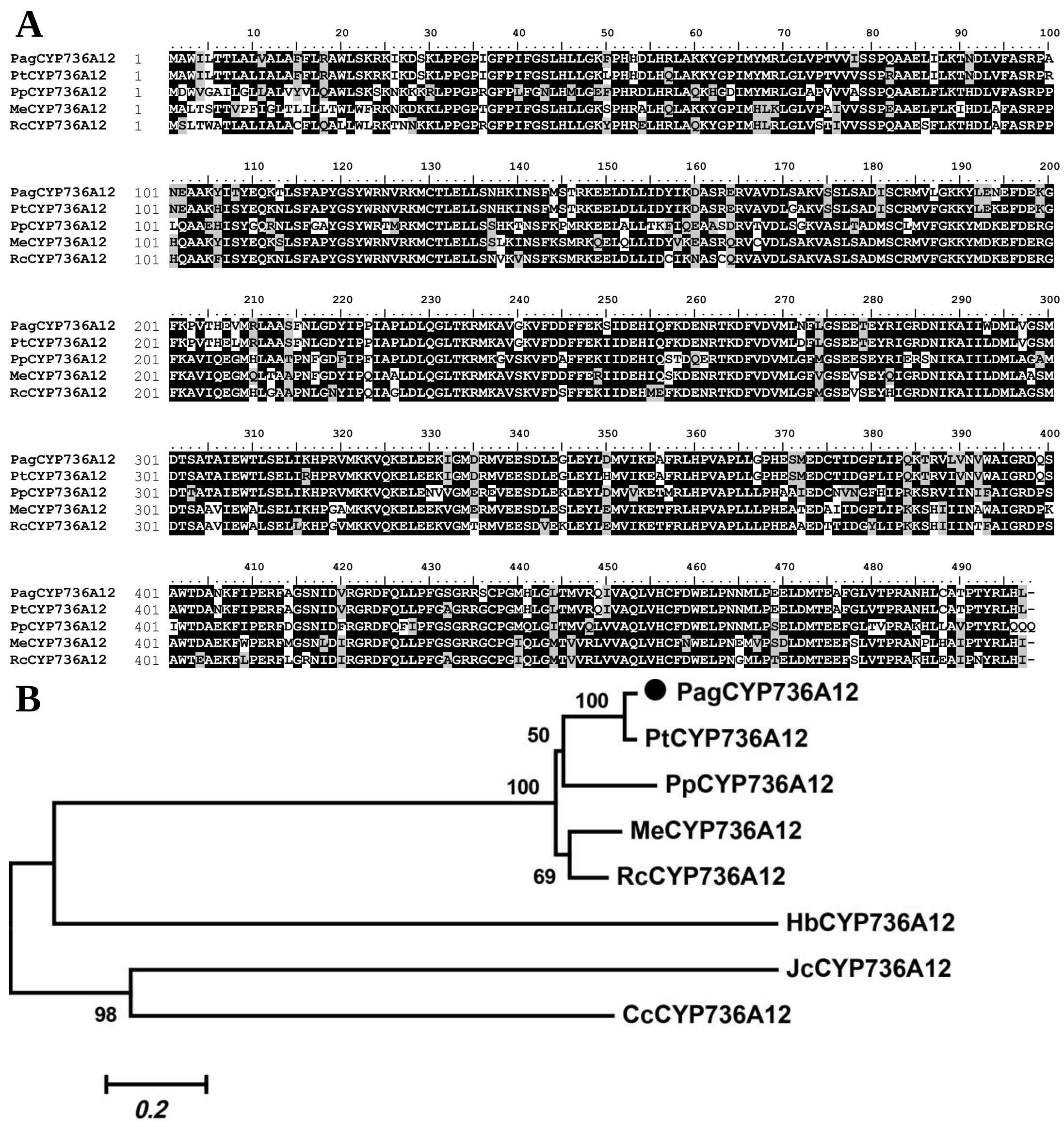

Figure S3 Sequence alignment and phylogenetic analysis of PagCYP736A12 and other plant CYP736A12 proteins. (A) Alignment of the amino acid sequences. The conserved amino acids are indicated by shading. (B) Phylogenetic tree of CYP736A12 proteins. The phylogram in which the branch lengths are proportional to sequence divergence was conducted with MEGA 5.05 using the neighbor-joining method. The numbers beside each node represent bootstrap values based on 1,000 replications. All amino acid sequences were retrieved from GenBank: *Populus alba* × *P. glandulosa* PagCYP736A12, *P. trichocarpa* (XP_002310014.2), *Manihot esculenta* MeCYP736A12 (XP_021630149.1), *Ricinus communis* RcCYP736A12 (XP_002532045.2), *Hevea brasiliensis* HbCYP736A12 (XP_021689116.1), *Jatropha* *curcas* JcCYP736A12 (XP_012075327.1), *Prunus persica* PpCYP736A12 (XP_007208656.1), and *Citrus clementina* CcCYP736A12 (XP_006428071.1).

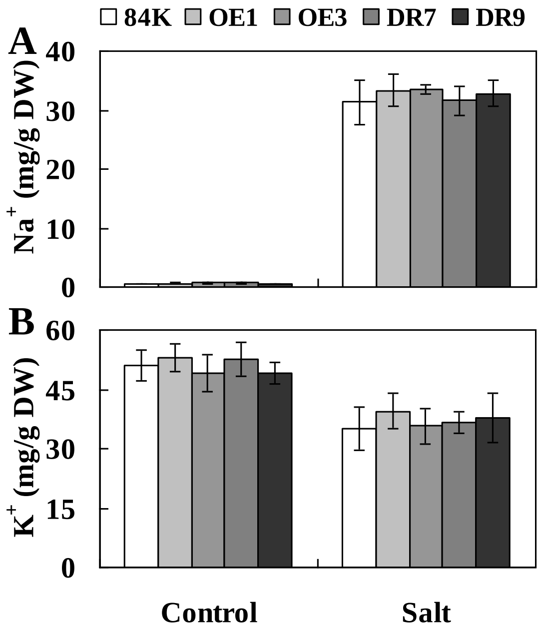

Figure S4 Na^+ and K^+ content in the roots of 84K and transgenic poplar plants. The cutting-propagated non-transgenic 84K and transgenic (OE and DR) poplar plants were cultivated and salinity treated with 0 and 75 mM NaCl as described in Figure 2. The roots were collected and used for analysis of ion content. DW, dry weight. The experiments were performed three times and the similar results were obtained. The bars represent means ± SD (n = 6) and the asterisks indicate significant differences between non-transgenic 84K and transgenic plants.

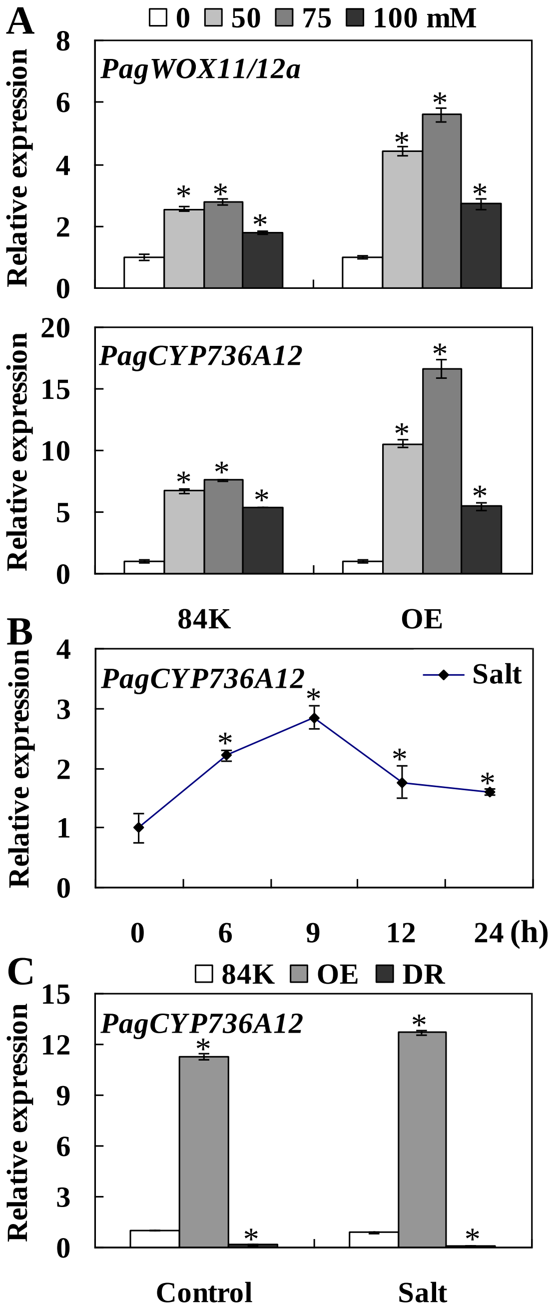

Figure S5 RT-qPCR analysis of the expression of *PagWOX11/12a* and *PagCYP736A12* in 84K and transgenic poplars. (A) The transcript levels of *PagWOX11/12a* and *PagCYP736A12* in roots from 84K and OE plants treated with different concentrations of NaCl. (B) The *PagCYP736A12* expression in total roots of poplar treated with 200 mM NaCl for the indicated times as described in Figure 1A. (C) The expression of *PagCYP736A12* in roots from 84K, OE, and DR plants tread with 0 and 75 mM NaCl. Poplar plants were cultivated and salinity treated with different concentrations of NaCl as described in Figure 2. The root tissues were collected from 10 individuals and used for RT-qPCR analysis. The experiments were performed three times and the similar results were obtained. The bars represent means ± SD (n = 4) and the asterisks indicate significant differences (* $P<0.05$).

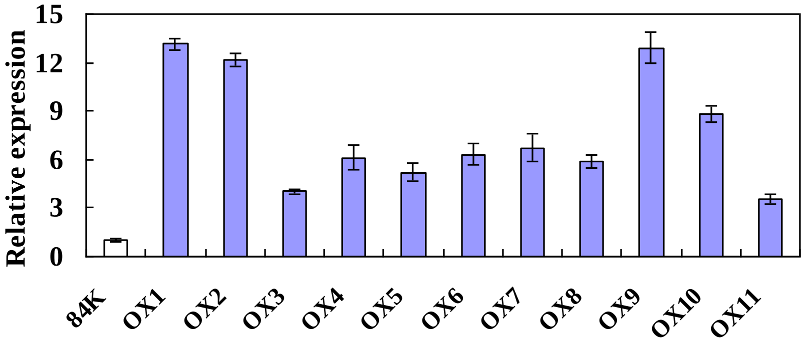

Figure S6 RT-qPCR analysis of *PagCYP736A12*-transformed poplars. RT-qPCR results showing the *PagCYP736A12* expression in 11 transgenic overexpression poplar lines (OX). *PagActin* genes were used as a reference gene for normalization. The bars represent means ± SD (n = 4).