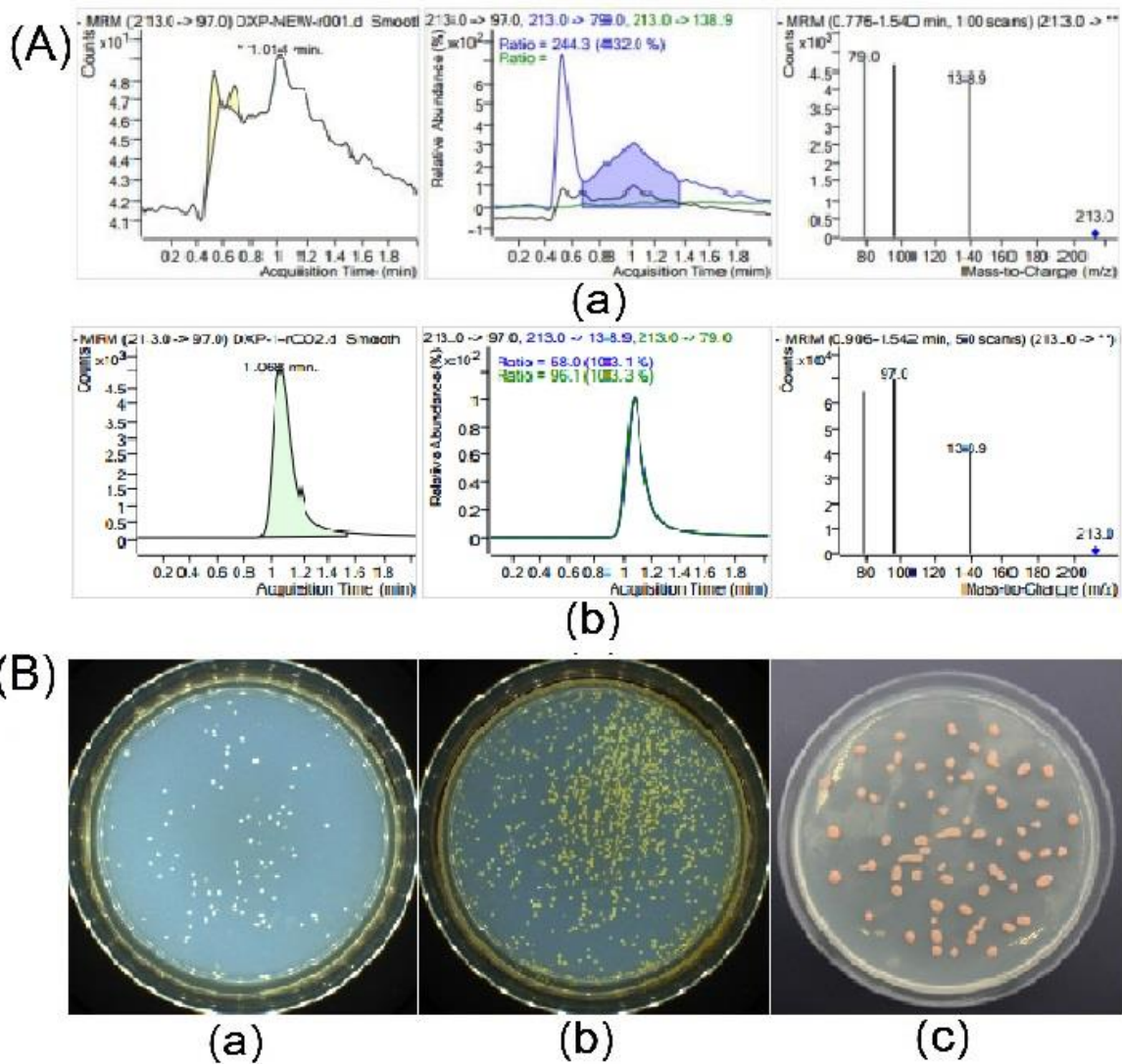
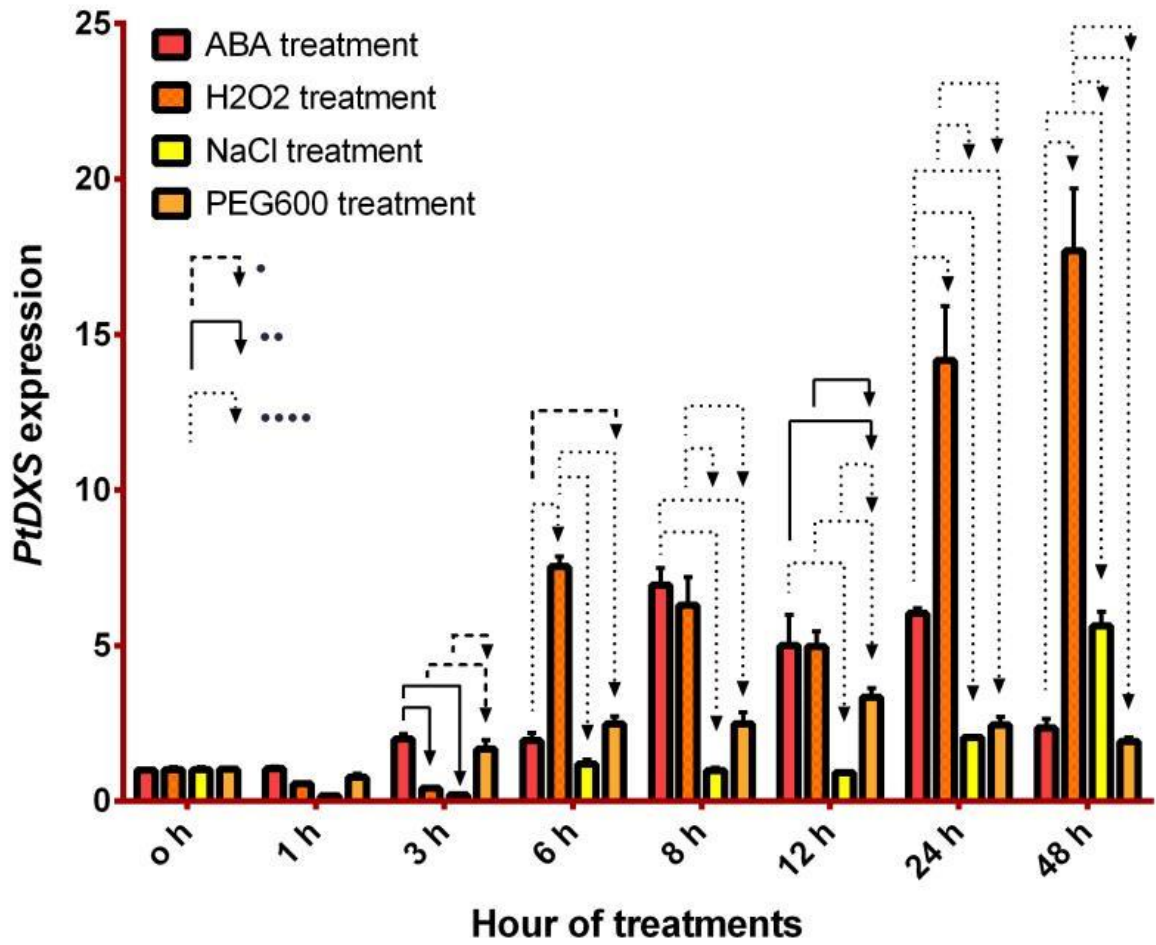


		-VXA-
<i>Populus trichocarpa</i>	1	MALSAFSLPAHNSVTS-LDHLQNSYFLSSFLWRDILGQSFKRINQYHVKKRPNVCAVLSSEGEPTSRQPPTPLDITNYPITHMKNLSIKELKQLAE
<i>Alpinia officinale</i>	1	MALFAFPFPHHLYGTA--ASNHQKATS-SPELITE---LHHRQEHSGSLRPKKRSSCVCAVLSSEIGEYHSQRPPPTPLDITNYPITHMKNLSIKELKQLAE
<i>Theobroma cacao</i>	1	MALCAFSFPAHNNVAA--ASDFQKSTSFASFLGGTDLDFPHLKNQYVKKRPNVCAVLSERAEYHSQRPPPTPLDITNYPITHMKNLSIKELKQLAD
<i>Hevea brasiliensis</i>	1	MALSAFSLPAHNDVAT--ISDLQKYGVYPSLSLWRDILGQSLGRINQAKSKKRFVGVCAVLSERGEYHSQRPPPTPLDITNYPITHMKNLSIKELKQLAD
<i>Ricinus communis</i>	1	MALCAFSFPAHNNVITASSDLQKSNVYSSNFLKTDLLGSSLQKFNQAKKKRPNVCAVLSSEIGEYHSQRPPPTPLDITNYPITHMKNLSIKELKQLAD
<i>Populus trichocarpa</i>	100	ELRSDVIFNYSKTTGGHLGSSLGVVELTVALHYVFNAPQDKILVDVGHQAVPHKILTGRRDKMVTTRQTNGLAGFTKRSESEYDCFGTGHSSSTISAGLGM
<i>Alpinia officinale</i>	95	ELRSDVIFNYSKTTGGHLGSSLGVVELTVALHYVFNAPQDKILVDVGHQAVPHKILTGRRDKMVTTRQTNGLAGFTKRSESEYDCFGTGHSSSTISAGLGM
<i>Theobroma cacao</i>	99	ELRSDVIFNYSKTTGGHLGSSLGVVELTVALHYVFNAPQDKILVDVGHQAVPHKILTGRRDKMVTTRQTNGLAGFTKRSESEYDCFGTGHSSSTISAGLGM
<i>Hevea brasiliensis</i>	99	ELRSDVIFNYSKTTGGHLGSSLGVVELTVALHYVFNAPQDKILVDVGHQAVPHKILTGRRDKMVTTRQTNGLAGFTKRSESEYDCFGTGHSSSTISAGLGM
<i>Ricinus communis</i>	101	ELRSDVIFNYSKTTGGHLGSSLGVVELTVALHYVFNAPQDKILVDVGHQAVPHKILTGRRDKMVTTRQTNGLAGFTKRSESEYDCFGTGHSSSTISAGLGM
		TPP motif
<i>Populus trichocarpa</i>	200	AVGRDLKGRNNVAVIGDGAMTAGQAYEAMNAGYLDSDMIVILNDNKQVSLPTANLDGPIPPVGLSSALSRLQSNRPLRELREVAKVTKQIGGPMH
<i>Alpinia officinale</i>	195	AVGRDLKGRNNVAVIGDGAMTAGQAYEAMNAGYLDSDMIVILNDNKQVSLPTANLDGPIPPVGLSSALSRLQSNRPLRELREVAKVTKQIGGPMH
<i>Theobroma cacao</i>	199	AVGRDLKGRNNVAVIGDGAMTAGQAYEAMNAGYLDSDMIVILNDNKQVSLPTANLDGPIPPVGLSSALSRLQSNRPLRELREVAKVTKQIGGPMH
<i>Hevea brasiliensis</i>	199	AVGRDLKGRNNVAVIGDGAMTAGQAYEAMNAGYLDSDMIVILNDNKQVSLPTANLDGPIPPVGLSSALSRLQSNRPLRELREVAKVTKQIGGPMH
<i>Ricinus communis</i>	201	AVGRDLKGRNNVAVIGDGAMTAGQAYEAMNAGYLDSDMIVILNDNKQVSLPTANLDGPIPPVGLSSALSRLQSNRPLRELREVAKVTKQIGGPMH
<i>Populus trichocarpa</i>	300	EIAAKVDEYARGMISGSGSTLFEELGLYYIGPVDGHNIDDLVAILKEVKSTKTTGPVLIHVVTEKGRGYPYAERAADKYHGVNKFDPATGKQKFSASTQ
<i>Alpinia officinale</i>	295	EIAAKVDEYARGMISGSGSTLFEELGLYYIGPVDGHNIDDLVAILKEVKSTKTTGPVLIHVVTEKGRGYPYAERAADKYHGVNKFDPATGKQKFSASTQ
<i>Theobroma cacao</i>	299	EIAAKVDEYARGMISGSGSTLFEELGLYYIGPVDGHNIDDLVAILKEVKSTKTTGPVLIHVVTEKGRGYPYAERAADKYHGVNKFDPATGKQKFSASTQ
<i>Hevea brasiliensis</i>	299	EIAAKVDEYARGMISGSGSTLFEELGLYYIGPVDGHNIDDLVAILKEVKSTKTTGPVLIHVVTEKGRGYPYAERAADKYHGVNKFDPATGKQKFSASTQ
<i>Ricinus communis</i>	301	EIAAKVDEYARGMISGSGSTLFEELGLYYIGPVDGHNIDDLVAILKEVKSTKTTGPVLIHVVTEKGRGYPYAERAADKYHGVNKFDPATGKQKFSASTQ
<i>Populus trichocarpa</i>	400	SYTTYFAEALIAEAEDKDIIVATHAAMGGGTGLNLFRRFPTRCFDVGIAEQHAVTFAAGLACEGLKPFCAYSSFMQRAYDQVVDVLDLQKIPVRFAMD
<i>Alpinia officinale</i>	395	SYTTYFAEALIAEAEDKDIIVATHAAMGGGTGLNLFRRFPTRCFDVGIAEQHAVTFAAGLACEGLKPFCAYSSFMQRAYDQVVDVLDLQKIPVRFAMD
<i>Theobroma cacao</i>	399	SYTTYFAEALIAEAEDKDIIVATHAAMGGGTGLNLFRRFPTRCFDVGIAEQHAVTFAAGLACEGLKPFCAYSSFMQRAYDQVVDVLDLQKIPVRFAMD
<i>Hevea brasiliensis</i>	399	SYTTYFAEALIAEAEDKDIIVATHAAMGGGTGLNLFRRFPTRCFDVGIAEQHAVTFAAGLACEGLKPFCAYSSFMQRAYDQVVDVLDLQKIPVRFAMD
<i>Ricinus communis</i>	401	SYTTYFAEALIAEAEDKDIIVATHAAMGGGTGLNLFRRFPTRCFDVGIAEQHAVTFAAGLACEGLKPFCAYSSFMQRAYDQVVDVLDLQKIPVRFAMD
		-DRAG- NADH-binding motif PSD-
<i>Populus trichocarpa</i>	500	RAG_VGADGPTHCGAFDVTFMACLPNMVMVPSDEAELFHMVATAAIDDRPSCFRYPNGVGVQLPPGNKGIPLEVGGKRIIEGERVALLGYGTAVQ
<i>Alpinia officinale</i>	495	RAG_VGADGPTHCGAFDVTFMACLPNMVMVPSDEAELFHMVATAAIDDRPSCFRYPNGVGVQLPPGNKGIPLEVGGKRIIEGERVALLGYGTAVQ
<i>Theobroma cacao</i>	499	RAG_VGADGPTHCGAFDVTFMACLPNMVMVPSDEAELFHMVATAAIDDRPSCFRYPNGVGVQLPPGNKGIPLEVGGKRIIEGERVALLGYGTAVQ
<i>Hevea brasiliensis</i>	499	RAG_VGADGPTHCGAFDVTFMACLPNMVMVPSDEAELFHMVATAAIDDRPSCFRYPNGVGVQLPPGNKGIPLEVGGKRIIEGERVALLGYGTAVQ
<i>Ricinus communis</i>	501	RAG_VGADGPTHCGAFDVTFMACLPNMVMVPSDEAELFHMVATAAIDDRPSCFRYPNGVGVQLPPGNKGIPLEVGGKRIIEGERVALLGYGTAVQ
<i>Populus trichocarpa</i>	600	SCLAAASLVERHGLHITVADARFCKPLDHALIRSLAKSHEVLITVEEGSIGGFGSHVAHFALDGLLDGKIKWRPVLDPDRYIDHGSPADQLIEAGLTPS
<i>Alpinia officinale</i>	595	SCLAAASLVERHGLHITVADARFCKPLDHALIRSLAKSHEVLITVEEGSIGGFGSHVAHFALDGLLDGKIKWRPVLDPDRYIDHGSPADQLIEAGLTPS
<i>Theobroma cacao</i>	599	SCLAAASLVERHGLHITVADARFCKPLDHALIRSLAKSHEVLITVEEGSIGGFGSHVAHFALDGLLDGKIKWRPVLDPDRYIDHGSPADQLIEAGLTPS
<i>Hevea brasiliensis</i>	599	SCLAAASLVERHGLHITVADARFCKPLDHALIRSLAKSHEVLITVEEGSIGGFGSHVAHFALDGLLDGKIKWRPVLDPDRYIDHGSPADQLIEAGLTPS
<i>Ricinus communis</i>	601	SCLAAASLVERHGLHITVADARFCKPLDHALIRSLAKSHEVLITVEEGSIGGFGSHVAHFALDGLLDGKIKWRPVLDPDRYIDHGSPADQLIEAGLTPS
<i>Populus trichocarpa</i>	700	HIAATVFNILGQRNSLIMSS
<i>Alpinia officinale</i>	695	HIAATVFNILGQRNSLIMSS
<i>Theobroma cacao</i>	699	HIAATVFNILGQRNSLIMSS
<i>Hevea brasiliensis</i>	699	HIAATVFNILGQRNSLIMSS
<i>Ricinus communis</i>	701	HIAATVFNILGQRNSLIMSS

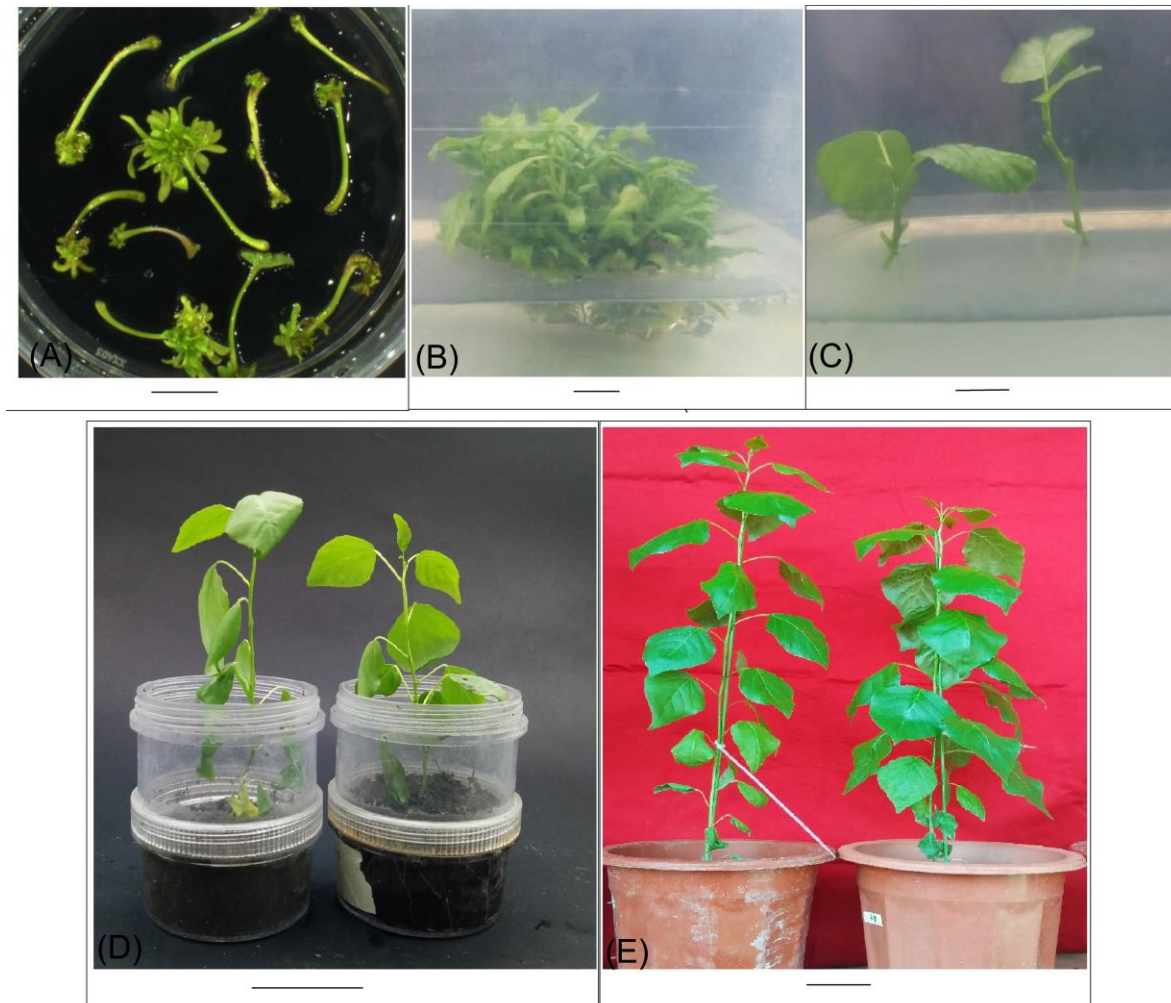
Supplemental figure 2. Deduced amino acid sequences of the conserved regions of *P. trichocarpa* *PtDXS* and of other *DXS* genes. The TPP motif and DRAG domain are numbered and indicated in the box.



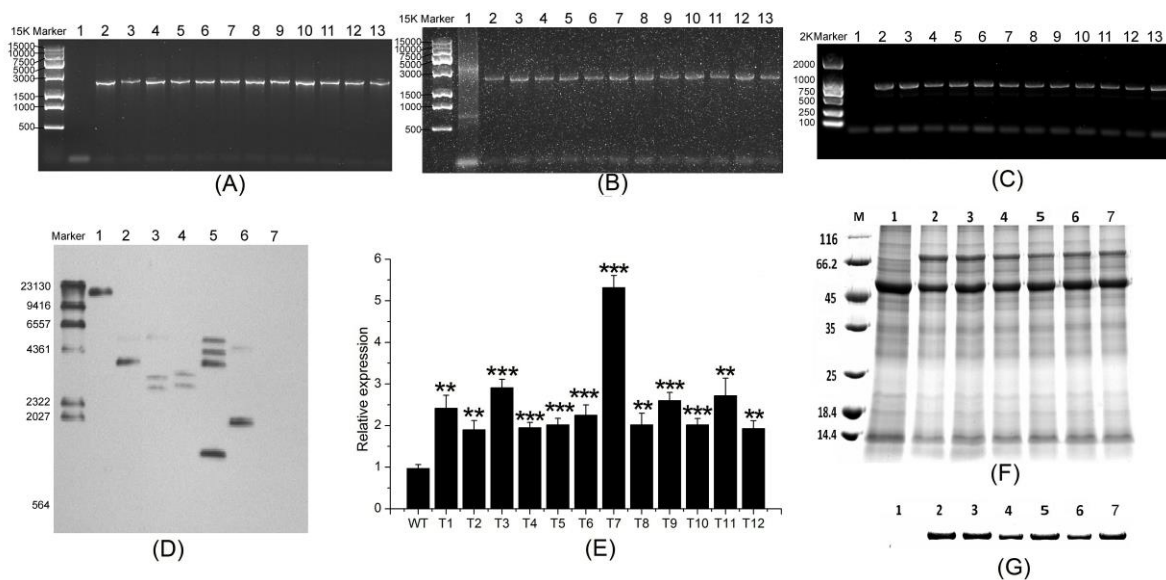
Supplemental figure 3. PtDXS activity in vitro and in vivo. (A) HPLC-MS/MS analysis of DXP (negative control group [a] and sample group [b]). (B) Functional analysis of PtDXS in *E. coli*. Single transformation of pTrc was performed as in [a]. *E. coli* cells harboring pTrc and pAC-BETA were treated as in [b]. *E. coli* cells harboring pTrc-PtDXS and pAC-BETA were treated as in [c].



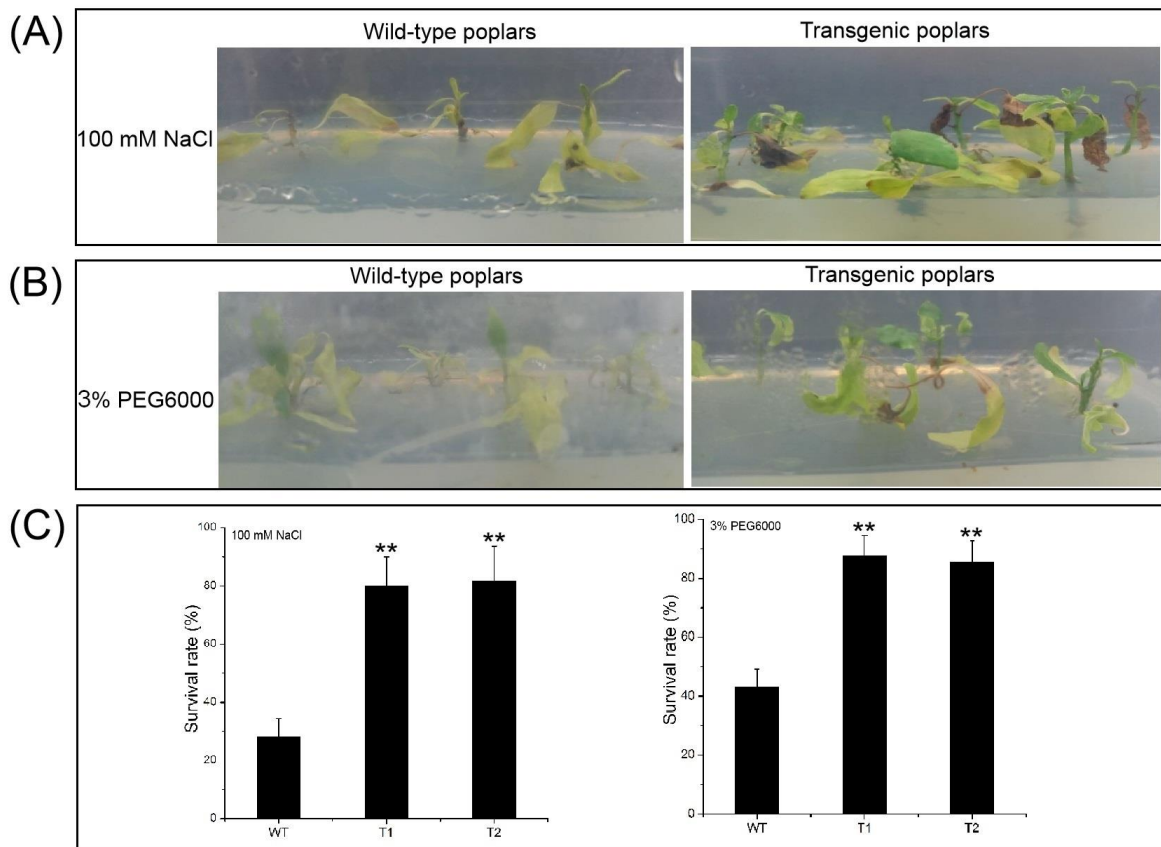
Supplemental Figure 4. Two-way ANOVA has been used to analyze the interaction between different abiotic stresses on *PtDXS* gene expression. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.0001$, respectively.



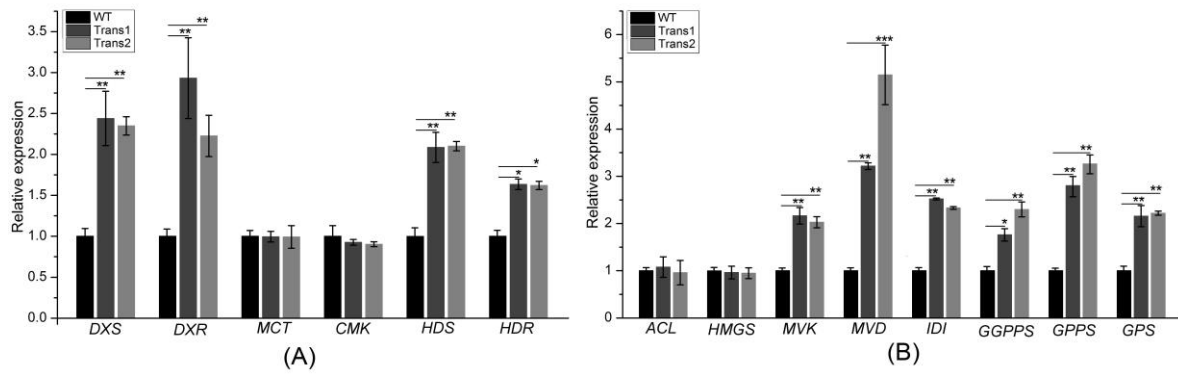
Supplemental figure 5. Regeneration of transformed explants on selective MS medium and regeneration of transgenic poplars in soil. (A) After co-cultivation of leaf pieces and petioles with *Agrobacterium* EHA105 containing *PtDXS*, transformed poplar plants were selected on regeneration medium supplemented with 30 mg/mL kanamycin. (B) Shoots were selected on bud elongation medium supplemented with 20 mg/mL kanamycin. (C) Roots were selected on half-strength MS medium supplemented with 10 mg/mL kanamycin. (D) and (E) Transgenic poplar seedlings grown in soil matrix. A and B Bar=1 cm. C Bar=1.5 cm. D Bar=3 cm. E Bar=6 cm.



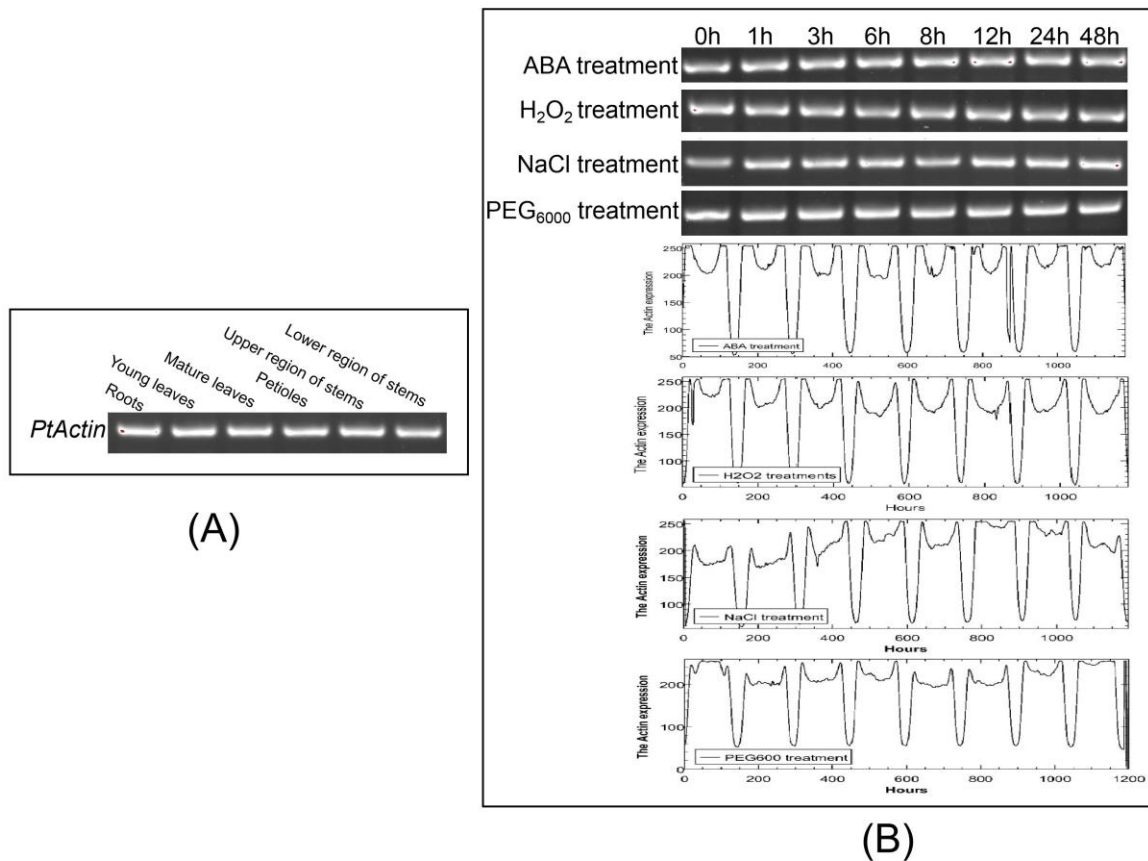
Supplemental figure 6. Identification of regenerated *PtDXS* transgenic poplar plants. (A) Detection of the *PtDXS* gene in transgenic lines and WT through PCR using the genome as template, the primer of the 35S promoter as the upstream primer, and the primer *PtDXS*-R as the downstream primer. Lane 1: negative control; lanes 2–13: transgenic lines 1–12 (T1-T12), respectively. (B) Detection of the *PtDXS* gene in transgenic lines and WT through PCR using the genome as template, the primer of the *PtDXS*-F as the upstream primer, and the primer *PtDXS*-R as the downstream primer. Lane 1: negative control; lanes 2–13: transgenic lines 1–12 (T1-T12), respectively. (C) Detection of the *kanamycin* gene in transgenic lines and WT through PCR using the genome as template, the primer of the *Kan*-F as the upstream primer, and the primer *Kan*-R as the downstream primer. Lane 1: negative control; lanes 2–13: transgenic lines 1–12 (T1-T12), respectively. (D) Southern blot analysis of transgenic poplars. Lane M: Marker. Lane 1: The vector PBGWB9-*PtDXS* digested with *EcoRI*. Lanes 2-6: Transgenic lines 1-5 (T1-T5) digested with *EcoRI*. Lanes 7: Wild-type poplars digested with *EcoRI*. (E) qRT-PCR analysis of *PtDXS* expression in WT and transgenic plants (T1-T12). (F) SDS-PAGE analysis of *PtDXS* levels in WT and transgenic plants. Lane M: protein markers; lanes 1–9: WT, T1, T2, T3, T7, T9, and T10 plants, respectively. (G) Western blot of *PtDXS* protein using a monoclonal antibody against the 6^{*}His tag. Lane M: Marker. Lane 1: Proteins from wild-type poplars. Lanes 2-7: Proteins from transgenic lines (T1, T2, T3, T7, T9, and T10), respectively.



Supplemental figure 7. Rooting abilities of wild-type and transgenic poplars under 100 mM NaCl and 3% PEG6000. (A) Phenotype of WT and transgenic poplars treated with 100 mM NaCl. (B) Phenotype of WT and transgenic poplars treated with 3% PEG6000. (C) Survival rates after 100 mM NaCl and 3% PEG6000 treatment. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.



Supplemental figure 8. Expression levels of MEP- and MVA-related genes in the transgenic poplars overexpressing *PtHMGR* and WT poplars (Control). (A) Expression levels of *DXS*, *DXR*, *MCT*, *CMK*, *HDS*, and *HDR*. (B) Expression levels of *ACL*, *HMGS*, *MVK*, *MVD*, *IDI*, *GPS*, *GPPS*, and *GGPPS*. Vertical bars represent means \pm SE ($n = 3$). Three independent experiments were performed. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.



Supplemental figure 9. The $\beta actin$ gene was selected as a reference gene. The $\beta actin$ expression in different organs of poplars and experimental conditions was investigated by RT-PCR. (A) The expression of $\beta actin$ in roots, young and mature leaves, petioles, and upper and lower regions of stems. (B) The expression of $\beta actin$ under the 200 μM ABA, 2 mM H₂O₂, 200 mM NaCl treatment and 10% PEG6000 treatments.