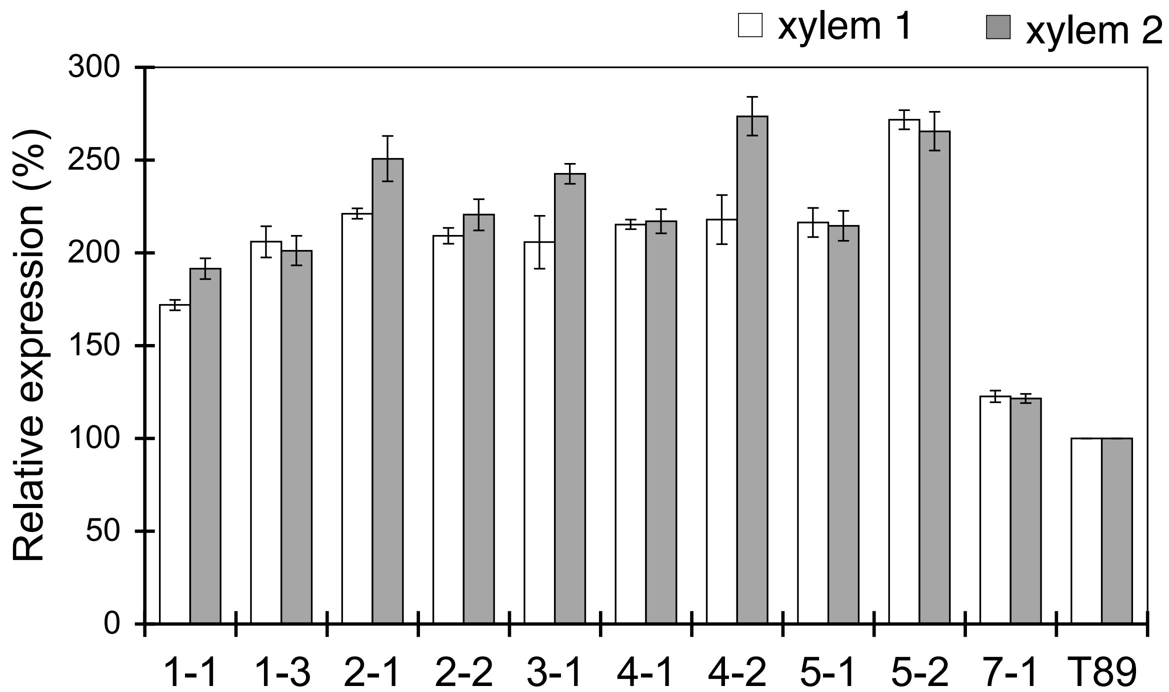

Figure S1.

PtxtXET16-34 transcript levels in the wood-forming tissues of *PtxtXET16-34* sense transgenic lines relative to WT (T89) levels as determined by RT-PCR.

After four months of growth in soil, total RNA from primary- and secondary-walled developing xylem of the 40th to 50th was extracted to analyze the expression level of *PtxtXET16-34* in each transgenic line by the competitive RT-PCR. Expression was first normalized to the 18S RNA level and then to the WT. Xylem 1 - primary-walled developing xylem tissue; Xylem 2 - secondary walled developing xylem tissue. Averages of three independent experiments ± SE.

Methods:

Relative quantitative RT-PCR

The selected transgenic lines and WT hybrid aspen were grown in a greenhouse for four months. Xylem 1 and 2 fractions were obtained as described above from internodes 40th to 50th. Total RNA was extracted with the RNeasy mini kit (Qiagen, Valencia, CA) either directly from the ground tissue or from crude RNA preparation obtained by the hot hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO) extraction procedure (Chang et al., 1993). RT was performed using the RetroScript kit (Ambion, Austin, TX), according to the manufacturer’s instructions with random primers and 1.7 μg of total RNA as template. *PtxtXET16-34* transcript quantities were determined by the semi-quantitative RT-PCR using 18S rRNA as internal control using QuantumRNA kit (Ambion, Austin, TX) according to the manufacturer's instructions. Gene-specific primer pair, capable of amplifying a 0.38-kb *PtxtXET16-34* fragment was XET16A-3UTR-F (AGTTCCAAGATCTGGATGCCTTC) and PtXET16A-rev (TGGTTCATTACAGTTTGACAACACAA). PCR cycle numbers were 25 (xylem 1) and 27 (xylem 2) in the OE lines, and 29 (xylem 1) and 34 (xylem 2) for RNAi lines. PCR products were analyzed on a 1.5% (w/v) agarose gel containing ethidium bromide and the signal intensities were determined with a Typhoon scanner 9600 (Amersham Biosciences) and Documentation System and Molecular Analyst software (Bio-Rad Laboratories, Hercules, CA). Each experiment was repeated at least three times using the same cDNA source, originating from pooled material of five to seven plants per genotype.

References:

Chang, S., Puryear, J. and Cairney, J. (1993) A simple and efficient method for isolating RNA from pine trees. *Plant Mol Biol Reporter,* **11**, 113-116.

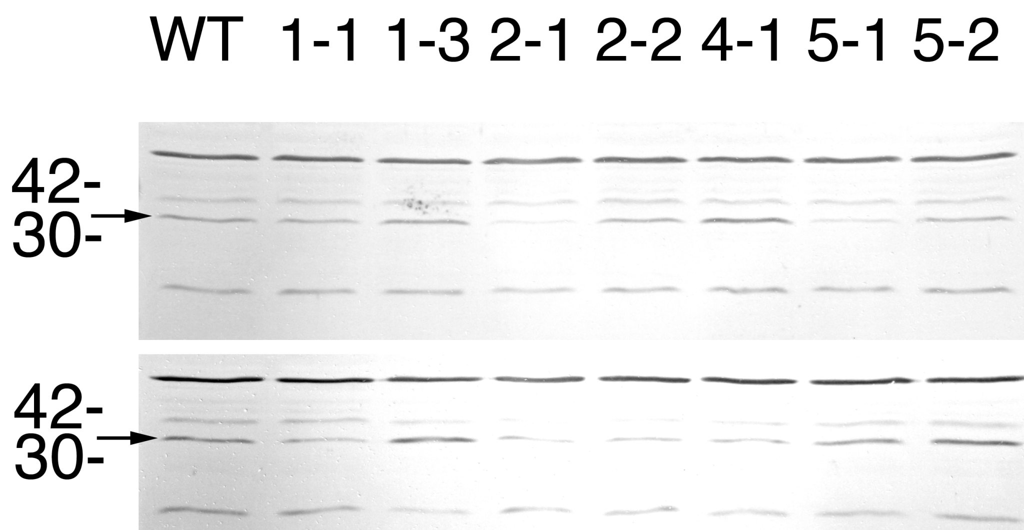

Figure S2.

Western blotting of proteins extracted form developing xylem tissues in *PtxtXET16-34*- overexpressing lines and the WT. XET16A antibody was raised against the recombinant *PtxtXET16-34* protein (Bourquin et al, 2002). Upper and lower panel represent primary- and secondary-walled developing xylem, respectively. The specific signal is indicated by the arrows. Other bands represent unspecific binding of the polyclonal antibody and visualize the variation in protein loading.

Proteins were extracted from primary- and secondary-walled developing xylem of the 40th to 50th internode tissues by incubation of the powdered tissue in Laemmli buffer. Primary XET16A antibody CHAD was used at 1:1000 dilution. Secondary antibodies conjugated to alkaline phosphatase were used for detection of the signal.

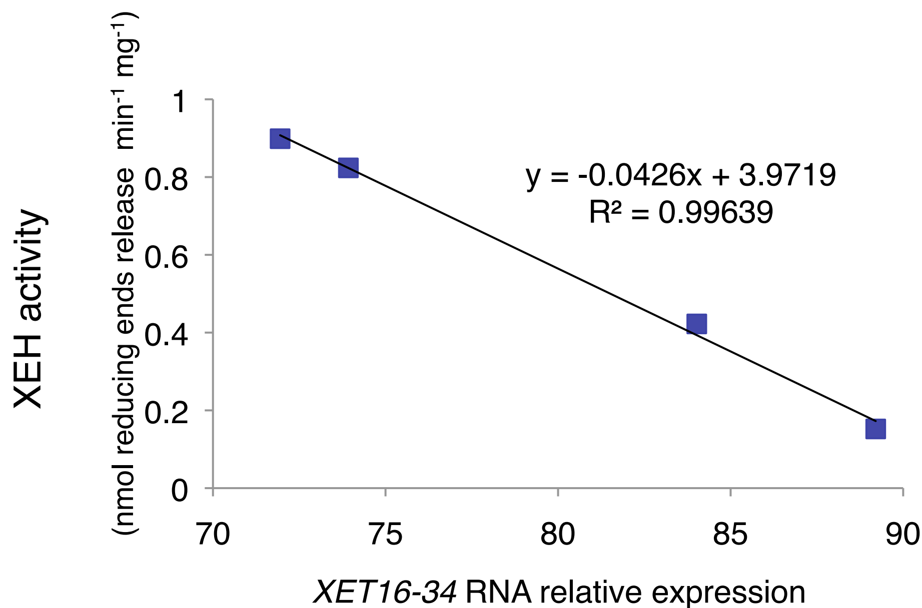

Figure S3.

Relationship between xyloglucan endo-hydrolase (XEH, EC= 3.2.1.151) activity and *PtxtXET16-34* expression level in secondary walled developing xylem of transgenic lines overexpressing *PtxtXET16-34*. The activity was measured in protein extracts as an increase in concentration of reducing sugars. *PtxtXET16-34* expression is relative to 18S RNA signal.

Xyloglucan endo-hydrolase activity assay

Primary- and secondary-walled xylem tissue fractions from internodes 40-50 of four to five trees representing each line were scraped into liquid nitrogen and ground. 100 mg of tissue powder was combined with 0.5 ml of ice-cold extraction buffer (50mM NaP buffer pH7, 2mM EDTA, 4% PVP mw 360 000, 1mM DTT). The samples were extracted at 4 °C for 30 min with shaking and then centrifuged for 15 min at 4 °C in a table centrifuge at maximum speed. The supernatants containing between 0.46 and 0.94 mg protein per ml, as determined by the Bradford assay, were collected. Xyloglucan endo-hydrolase activity in protein extracts was assayed by measuring reducing sugars by the Nelson-Somogyi procedure (Nelson, 1944; Somogyi, 1952). The tests were performed using 100 mg of xyloglucan (Megazyme) and 80µl of extracted protein according to Megazyme instruction. Reaction was carried out at 25°C overnight, stopped and absorbance was measured at 520nm. As a positive control 2 mg of xyloglucan endoglucanase (XEG) generously provided by Novozymes A/S, Denmark, were used instead of extracted proteins. Negative controls were prepared from proteins extract heated for 10 min at 95°C as well as by adding Nelson-Somogyi stopping solution to xyloglucan before adding proteins extract. The activity in triplicate samples of each line was measured, and their average specific activity was calculated in nanomoles of reducing ends per 1 mg of extracted protein per minute.

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem **153**: 375-381.

Somogyi M (1952) Notes on sugar determination. J Biol Chem **195**: 19-23.

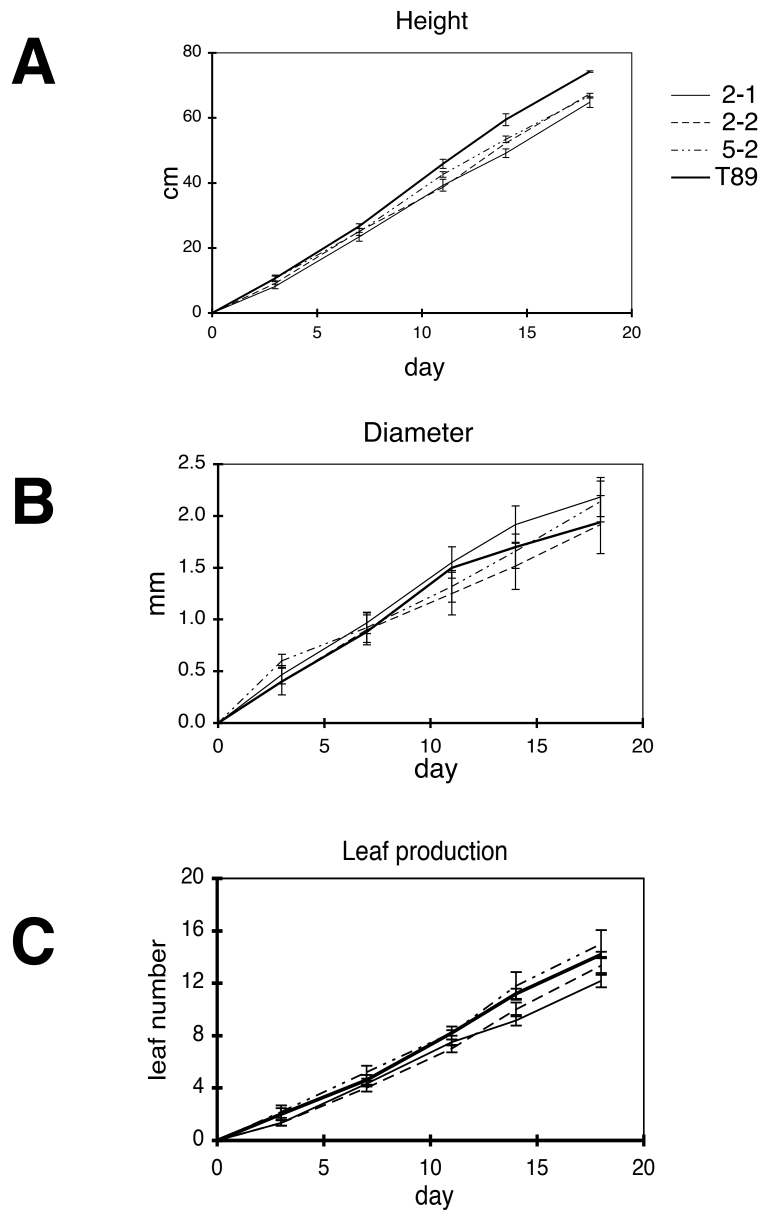

Figure S4.
Effects of *PtxtXET16A-34* overexpression on plant growth.

Overexpression of *PtxtXET16-34* in transgenic poplar slightly inhibited stem elongation (**A**) but the diameter growth (**B**) and the number of leaves (**C**) were not significantly changed. Plants were measured one month after potting. T89 - WT.

Table S1. *Populus XTH* gene models as identified by Geisler-Lee et al., 2006, updated to the P. trichocarpa genome version 1.1 (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html) and corresponding EST clones found in different tissue-specific libraries in the Swedish EST database (http://www.populus.db.umu.se/), or obtained by RT-PCR amplification from the cambial region mRNA using XET-specific degenerate primers (as indicated by the number after /).

XTH No	Alias	Accession numbers	Genemodel v1.1	protein ID	Location	EST clones/RT-PCR clones									
						cambial region	dormant cambium	tension wood	bark	apical bud	root	leaf	petiole	flower	seeds
1	S	EF194048	eugene3.14600001	582987	scaffold_1460:1-1079	0/1									
2			fgenesh4_pm.C_scaffold_131000027	811167	scaffold_131:426441-427561										
3	M	EF194053	grail3.0018003901	646298	LG_III:9672371-9675879			1					1		
4			eugene3.00660170	594976	scaffold_66:1165826-1166385										
5			eugene3.01570037	584043	scaffold_157:396919-398369										
6	E	EF194049	fgenesh4_pg.C_LG_VI000528	762218	LG_VI:4521539-4522594		5		1	1	7		1	2	9
7			fgenesh4_pg.C_LG_IX001489	768438	LG_IX:11581492-11583071										
8			eugene3.00021425	552110	LG_II:11596427-11597141										
9			eugene3.00040523	555810	LG_IV:5025762-5026321										
10			eugene3.00110488	568474	LG_XI:5120628-5121988										
11			estExt_Genewise1Plus.C_LG_XVIII23	1106803	LG_XVIII:9720722-9721882										
12			gw1.II.2740.1	411405	LG_II:4090484-4091490										2
13			estExt_Genewise1_v1.C_LG_V0594	714863	LG_V:13766983-13767994										
14	K	EF194054	eugene3.00140931	572886	LG_XIV:7223569-7225157	0/3				1		1		1	
15			e_gw1.II.270.1	851311	LG_II:22816722-22818310										
16			gw1.XI.1790.1	233810	LG_XI:2158121-2159708										
17	I	EF194056	estExt_Genewise1_v1.C_LG_XVIII2839	737795	LG_XVIII:9732450-9734010	0/4			2		2				2
18			estExt_Genewise1_v1.C_LG_XVIII2844	737800	LG_XVIII:9738560-9739859										
19			gw1.XVIII.2837.1	262296	LG_XVIII:9714098-9715068										
20			fgenesh4_pg.C_scaffold_171000033	786577	scaffold_171:422480-423527										
21	G	EF194058	estExt_Genewise1_v1.C_LG_II3808	711462	LG_II:22154322-22156560	0/24				2			1	1	6
22			fgenesh4_pg.C_LG_IX000065	767014	LG_IX:396662-397531										
23			fgenesh4_pg.C_LG_XVIII000708	779340	LG_XVIII:8565029-8566704										
24			eugene3.00110489	568475	LG_XI:5135299-5136430										
25	AA	EF194051	eugene3.00040079	555366	LG_IV:743969-746147									1	
26	B	EF194046	estExt_Genewise1_v1.C_LG_XIV2905	732010	LG_XIV:6764512-6766654	1/1									
27	R	EF194052	grail3.0008019801	640150	LG_I:5147426-5150019	0/7		1	2	2				3	
28			eugene3.00051083	559468	LG_V:13776227-13777443										
29	BB	EF194045	eugene3.00090819	557559	LG_IX:5040890-5042029	0/1									
30	H	EF194057	eugene3.00131219	571855	LG_XIII:12239791-12242484	10		1		1			1	9	1
31			eugene3.00160931	576796	LG_XVI:8835690-8838106									1	
32			eugene3.00280224	589488	scaffold_28:2448009-2449922										
33			eugene3.16810001	584844	scaffold_1681:5372-6543					1					
34	A[1]	AF515607	estExt_Genewise1_v1.C_LG_III1280	712151	LG_III:14324903-14327669	4/9		1		1			3	3	9
35	C	EF151160	eugene3.00190998	574524	LG_XIX:11359460-11362792	5/4		6		5				22	
36	D	EF194050	grail3.0019035302	655512	LG_VII:12024185-12043591				7	2		2	9	2	
37			eugene3.00130049	570685	LG_XIII:452955-454171										3
38	T	EF194047	estExt_Genewise1_v1.C_LG_XIV2164	731609	LG_XIV:4884130-4885664			3							
39	J	EF194055	estExt_Genewise1_v1.C_LG_VIII2102	720800	LG_VIII:9147474-9150429	1/2			1			2	1		
40	L[2]		grail3.0006015301	658681	LG_X:10736558-10738895	1								1	
41			eugene3.00290248	590065	scaffold_29:2684768-2687263										

[1] Bourquin et al., 2002

[2] Aspeborg et al., 2005