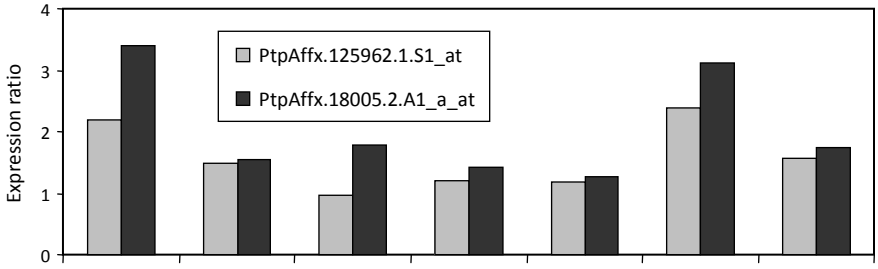
Table S1. Primer information

Gene	Forward and reverse primers	Purpose
GT1-316	F: GGATCCCTGGTTTCCGAGTCTCTTGG R: CCCGGGTCATGCCGATGACCCCAC	recombinant protein expression
GT1-316	F: ATGGTTTCCGAGTCTCTTGG R: TCATGCCGATGACCCCAC	binary vector construction
GT1-315/316 cluster	F: CCTAAGGCAGTGGAAATGAAGSAG R: CCAGTTCADCSACTTYCTCRHTGG	qRT-PCR
PAL1	F: ATGTCTTTGCTTACGCCGATGACC R: TCATATGCTGCTCTTGCGCTCTCA	qRT-PCR
PAL2	F: AGAGAGTCCTGACAATGGGCTTCA R: GYTGGGTTGCCRTTCTCAAKTTCA	qRT-PCR
4CL1	F: TAGTGAAATCAGAAAAGTCTCAGG R: CGCAAGTATTAAAGAAATAAGACAG	qRT-PCR
4CL2	F: TATTCCCAAATCGGCTTCTGG R: GGCAAGCTTGGCTCTCAGGTC	qRT-PCR
CCoAOMT1	F: RCCRATGAGGAAGTATGTGAGGTA R: CAGATAAYAATAYTGGCAGGTCCC	qRT-PCR
CCoAOMT2	F: RCCRATGAGGAAGTATGTGAGGTA R: TATAATATHGGCAGRGAATGCAGCCC	qRT-PCR
elongation factor 1-β	F: GACCTKGTATCAGTGGATTCCCTC R: GAACAGAGGCACAAGATTACCAGG	qRT-PCR
α-tubulin 4	F: CTTGCAATAGMATTCATCTGGGAAATGGT R: CTTCAAATACGAAGACAGACATAGTCTTG	qRT-PCR
ubiquitin-conjugating enzyme E2	F: CTGAAGAAGGAGATGACARCMCCA R: GCATCCCTTCAACACAGTTTCAMG	qRT-PCR

Supplemental Table 2. Characteristics of PfaGT1-316 glycosylation products confirmed by HPLC-MS/TOF.

Product name (substrate)	RT (min)	detected *m/z*	ion	expected *m/z*	Δ mass (ppm)	Product UV/Vis max	Substrate UV/Vis max	Molecular formula
caffeoyl-glucose (caffeic acid)	2.50	341.0865	$[M-H]^-$	341.0873	-2.35	335, 212, 300	323, 218, 294-ish, 235-240-ish	C15H18O9
2-coumaroyl-glucose (2-coumaric acid)	3.94	325.0934	$[M-H]^-$	325.0923	3.38	281, 330, 229, 218-ish	276, 324, 216	C15H18O8
4-coumaroyl-glucose (4-coumaric acid)	3.11	325.0929	$[M-H]^-$	325.0923	1.85	315, 228, 296-ish, 210-ish	310, 225, 290ish, 212ish	C15H18O8
4-hydroxybenzoyl-glucose (4-hydroxybenzoic acid)	1.73	299.0764	$[M-H]^-$	299.0767	-1.00	260, 210	256, 210ish	C13H16O8
sinapoyl-glucose (sinapic acid)	3.47	385.1126	$[M-H]^-$	385.1135	-2.34	331, 225, 240-ish	324, 225, 235-ish	C17H22O10
feruloyl-glucose (ferulic acid)	3.42	355.1042	$[M-H]^-$	355.1029	3.66	330, 220, 295-ish, 240-ish	322, 219, 295-ish, 232-ish	C16H20O9
cinnamoyl-glucose (cinnamic acid)	4.95	355.1043	$[M+CHOOH-H]^-$	355.1029	3.94	282	277, 218	C15H18O7
benzoyl-glucose (benzoic acid)	3.45	329.0888	$[M+CHOOH-H]^-$	329.0873	4.56	232, 276	228, 273	C13H16O7
naringenin glucoside* (naringenin)	5.58	433.1134	$[M-H]^-$	433.1135	-0.23	284, 223, 332-ish	288, 222, 327-ish	C21H22O10
4-hydroxybenzaldehyde glucoside* (4-hydroxybenzaldehyde)	1.79	329.0890	$[M+CHOOH-H]^-$	329.0873	5.17	265, 213, 220-ish, 280-ish	283, 220, 200, 268-ish	C13H16O7
kaempferol glucoside* (kaempferol)	5.54	447.0951	$[M-H]^-$	447.0927	5.37	364, 263, 249-ish, 315-ish	363, 265, 320-ish, 300-ish	C21H20O11

* The exact identity (the natural of the glycosidic linkage) of these metabolites was not verified.



Tissue	YL	EL	YL	EL	YL	EL	EL
Stress	1-wk N stress		1-wk N stress		1-wk post wounding		90-hr post detopping
Genotype	*P. fremontii x angustifolia* clone 1979		*P. fremontii x angustifolia* clone 3200		*P. fremontii x angustifolia* clone RM5		*P. tremuloides* clone 271

Fig. S1. Expression response of two *GT1-315/316* probe-sets to various stress treatments in multiple *Populus* genotypes. Expression ratio of stressed-to-unstressed samples was calculated using MAS5-processed Affymetrix microarrays, based on the mean of two biological replicates. YL, young leaves; EL, expanding leaves.

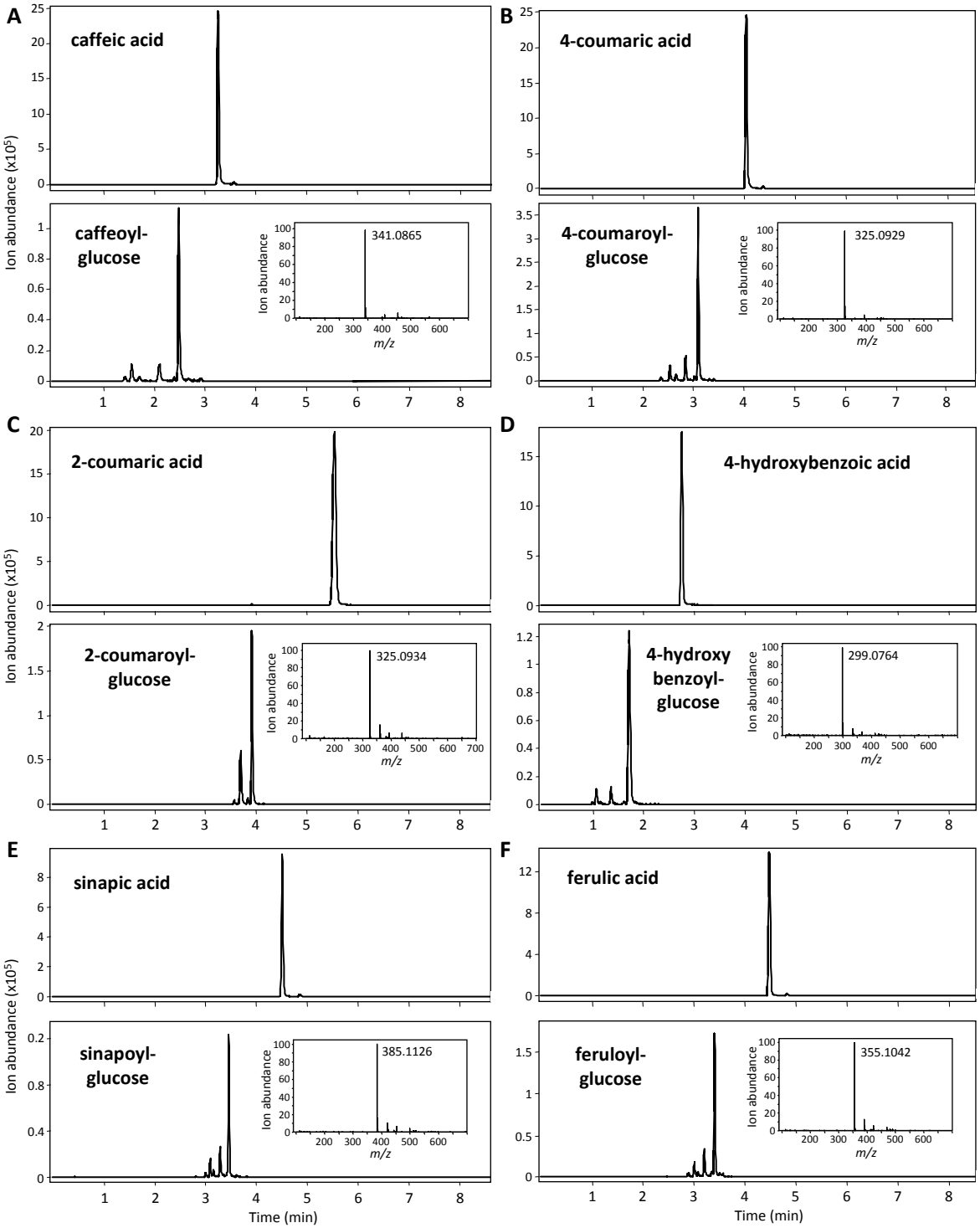


Fig. S2. HPLC-MS/TOF confirmation of PfaGT1-316 enzyme assay products. Extracted ion chromatograms for the substrate aglycone (top), glycosylation product (bottom) and mass spectra of the product (inset) are shown for (A) caffeic acid, (B) 4-coumaric acid, (C) 2-coumaric acid, (D) 4-hydroxybenzoic acid, (E) sinapic acid, (F) ferulic acid, (G) cinnamic acid, (H) benzoic acid, (I) naringenin, (J) 4-hydroxybenzaldehyde, (K) kaempferol. For each substrate there was one major product peak.

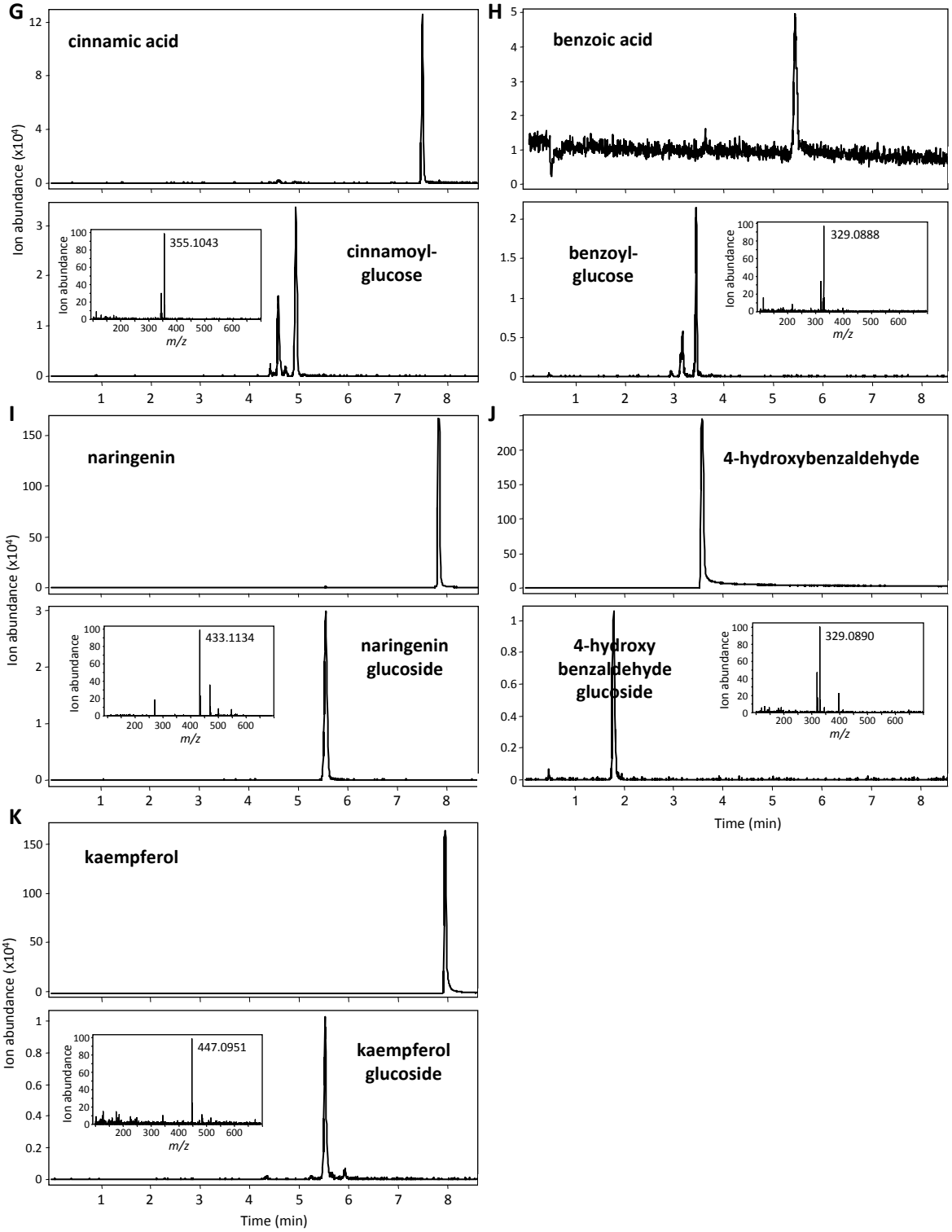


Fig. S2. (continued).

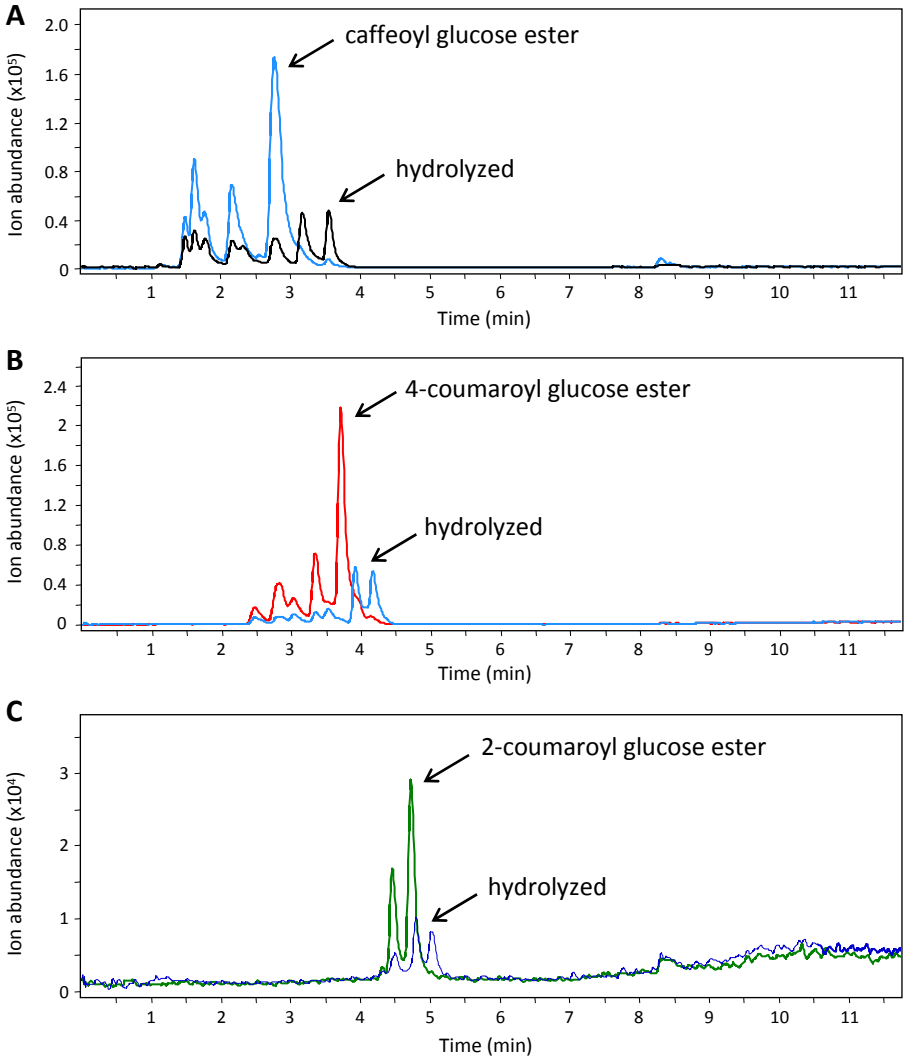


Fig. S3. Alkaline hydrolysis of PfaGT1-316 assay products to confirm glucose-ester linkage. Extracted ion chromatograms are shown for the major ion of glycosylation products of (A) caffeic acid, (B) 4-coumaric acid, and (C) 2-coumaric acid, with NaOH-hydrolyzed product chromatograms overlayed. Since the NaOH treatment cleaves only glucose ester, but not *O*-glucoside bonds, the digestibility of the PfaGT1-316 products strongly suggests that PfaGT1-316 catalyzes the formation of phenolic glucose esters, rather than phenolic *O*-glucosides.

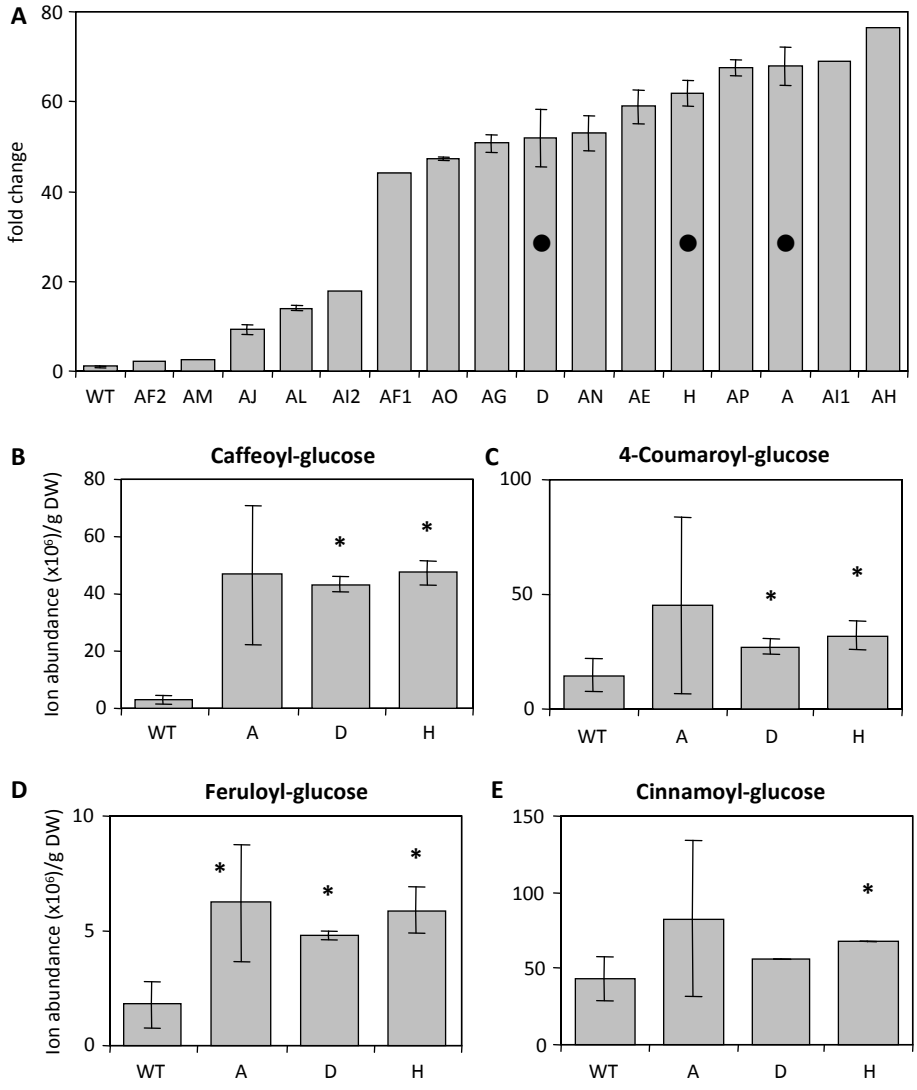


Fig. S4. Screening of independent GT1-316 transgenic lines. (A) qRT-PCR analysis of *GT1-315/316* transcript abundance in leaves (LPI-5) of WT and 16 independent primary transformants (n = 2 where error bars are shown). Lines marked with dots were selected for further analysis. (B) to (E), HPLC-MS/TOF analysis of caffeoyl- (B), 4-coumaroyl- (C), feruloyl- (D) and cinnamoyl-glucose esters (E) in WT and three transgenic lines. Statistical significance ($P < 0.05$) is marked by asterisks.

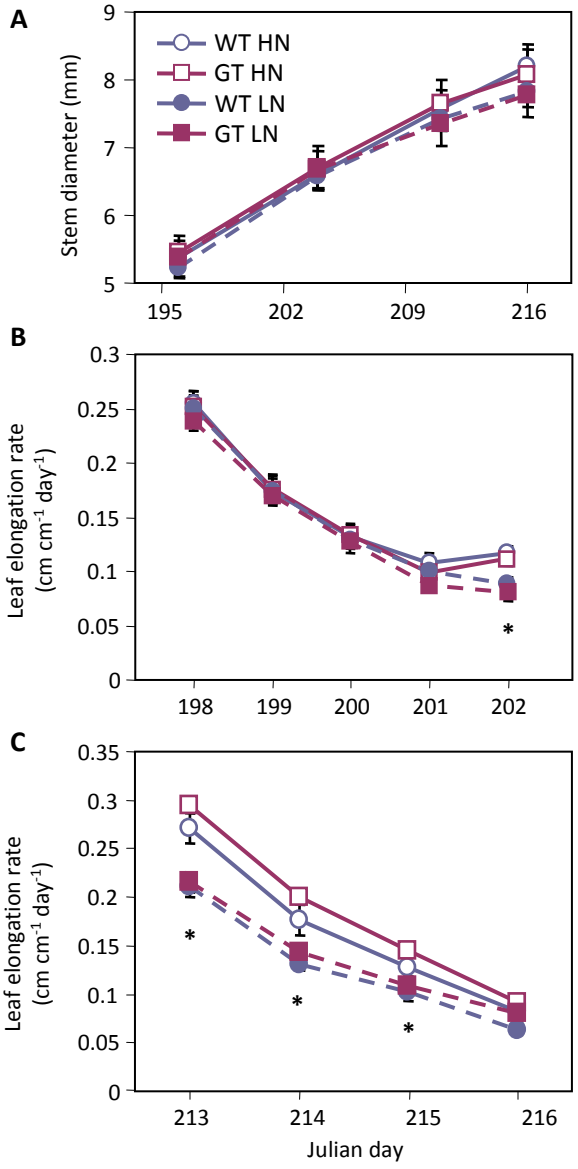


Fig. S5. Additional growth data. (A) Stem diameter and (B)-(C) leaf elongation rate at the onset (B) and after two weeks (C) of low-N treatments in WT and PfaGT1-316 over-expressing transgenic (GT) plants. Data represent means and standard errors of n= 15 in (A) and n= 5-6 in (B) and (C). N treatment effects are indicated by asterisks ($P < 0.05$).

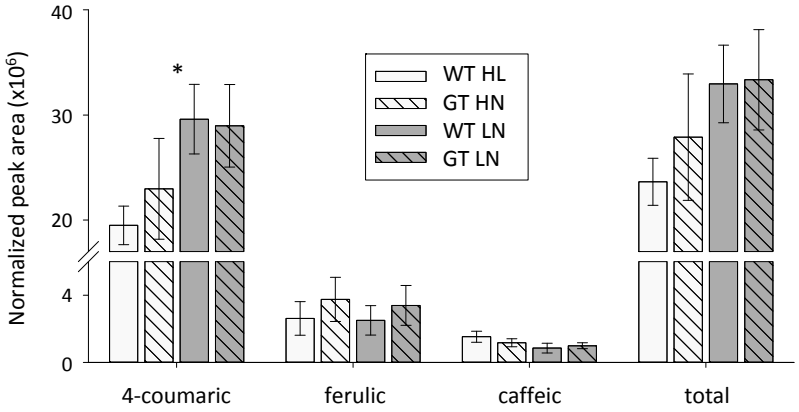


Fig. S6. Analysis of wall-bound phenolics in xylem of WT and transgenic *Populus* grown under different N regimes. There was no statistically significant transgenic effect. N treatment effects were indicated by an asterisk ($P < 0.05$).

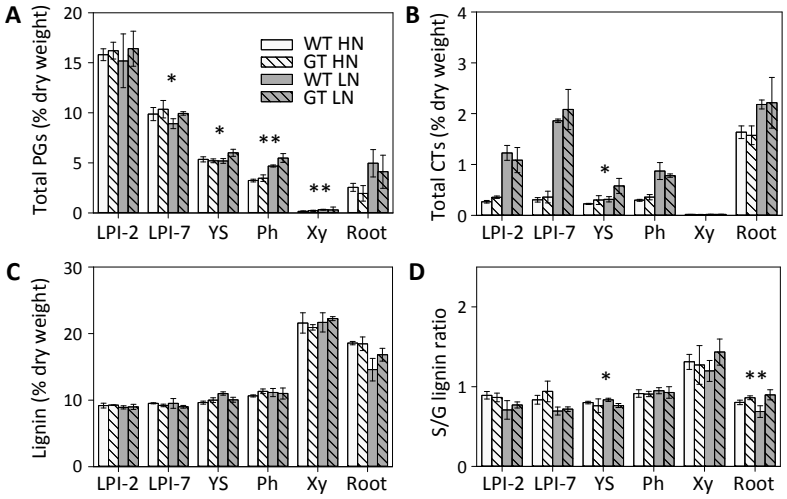


Fig. S7. Effects of PfaGT1-316 over-expression on major phenylpropanoid products. Shown are (A) phenolic glycoside (PG), (B), condensed tannin (CT), and (C) lignin content, and (D) S/G lignin ratio. Bars indicate means ± SE (n= 3). Asterisks indicate significant genotypic differences from two way ANOVA (genotype × N treatment); * $P < 0.1$; ** $P < 0.05$.

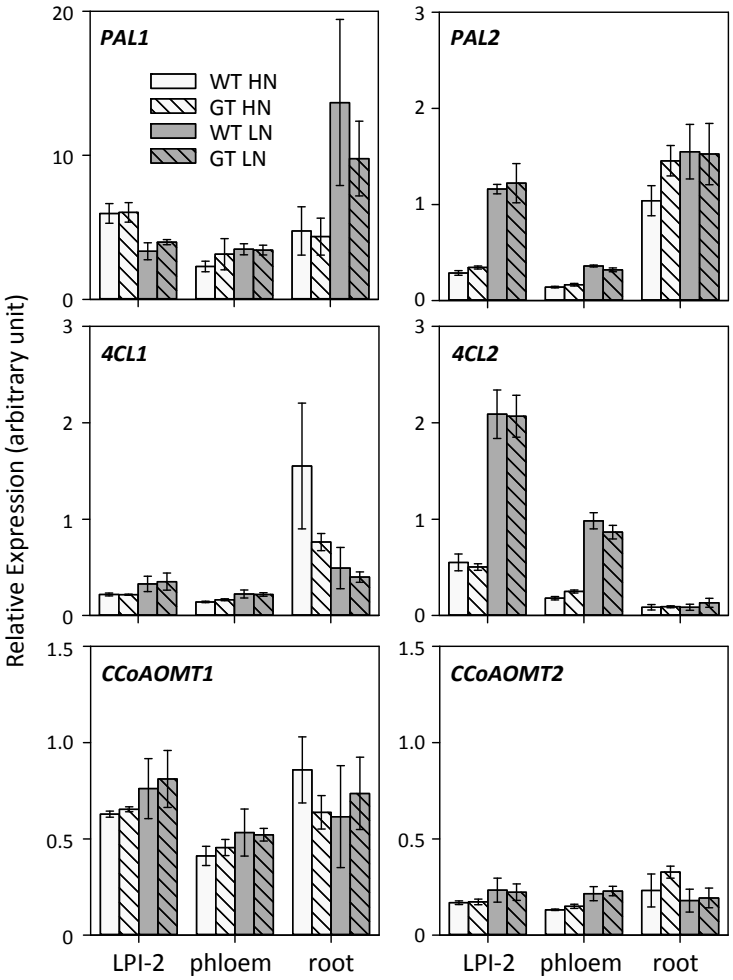


Fig. S8. Relative transcript abundance of representative phenylpropanoid pathway genes in WT and transgenic *Populus* grown under different N regimes. Two isoforms each for PAL (phenylalanine ammonia lyase), 4CL (4-coumarate:CoA ligase) and CCoAOMT (caffeoyl-CoA 3-*O*-methyltransferase) were analyzed. Numbering of isoforms is based on Tsai *et al*. (2006).

Tsai CJ, Harding SA, Tschaplinski TJ, Lindroth RL, Yuan Y. 2006. Genome-wide analysis of the structural genes regulating defense phenylpropanoid metabolism in *Populus*. New Phytologist 172, 47-62.