Gene	Forward and reverse primers	Purpose	
TUA1	F: GC <u>TCTAGA</u> TGAGAGAGTGCATTTCGATTCA R: TCC <u>CCCGGG</u> TCAGTACTCATCTCCTTCAT	Cloning	
TUA1dY	F: GCTCTAGATGAGAGAGTGCATTTCGATTCA R: ACGC <u>GTCGAC</u> TCACTCATCTCCTTCATCACCAT	Cloning	
TUA1dEY	F: GCTCTAGATGAGAGAGTGCATTTCGATTCA R: ACGC <u>GTCGAC</u> TCAATCTCCTTCATCACCATCCT	Cloning	
TUB9	F: GC <u>TCTAGA</u> TGAGAGAAATCCTTCATG R: TCC <u>CCCGGG</u> TTAGTTCTCCATAGGCTCTT	Cloning	
TUB15	F: GC <u>TCTAGA</u> TGAGAGAAATCCTTCACATTC R: TCC <u>CCCGGG</u> CTAGGCAGCCTCTTCCTCCT	Cloning	
TUA1dY/TUA1dEY transgenes	F: GCTCTAGATGAGAGAGTGCATTTCGATTCA R: ATCGCAAGACCGGCAACAGG	genomic PCR	
TUB9 transgene	F: GCTCTAGATGAGAGAAATCCTTCATG R: ATCGCAAGACCGGCAACAGG	genomic PCR	
TUB15 transgene	F: GCTCTAGATGAGAGAAATCCTTCACATTC R: ATCGCAAGACCGGCAACAGG	genomic PCR	
Glycosyltransferase (housekeeping)	F: TTGGGCTTGAGCAAAGTGG R: AGATCCACCCTCCAACAGTG	genomic PCR	
НРТ	F: GAGGGCGAAGAATCTCGTGC R: GATGTTGGCGACCTCGTATTG	genomic PCR	
NPT	F: GAACAAGATGGATTGCACGC R: GAAGAACTCGTCAAGAAGGC	genomic PCR	
TUA1 endogene	F: CGATGGAGAGGATGGTGATGAAGGA R: CACGTACCAACAGACATGGTCTAAGC	qRT-PCR	
TUB9 endogene	F: CAGTCATGTTTAGGAGAAARGCGT R: CAACTCATCCTTGAAGCCATRGCC	qRT-PCR	
TUB15 endogene	F: ACAGCTATGTTCAGGAGGAAGGCT R: TCACACCAGCCACATGGCTTAMTA	qRT-PCR	
TUA1dY/TUA1dEY transgenes	F: CGATGGAGAGGATGGTGATGAAGGA R: TAATCATCGCAAGACCGGCAACAG	qRT-PCR	
TUB9 transgene	F: CAGTCATGTTTAGGAGAAARGCGT R: TAATCATCGCAAGACCGGCAACAG	qRT-PCR	
TUB15 transgene	F: ACAGCTATGTTCAGGAGGAAGGCT R: TAATCATCGCAAGACCGGCAACAG	qRT-PCR	
elongation factor 1-β (housekpeeing)	F: GACCTKGTATCAGTGGATTCCCTC R: GAACAGAGGCACAAGATTACCAGG	qRT-PCR	
actin (housekpeeing)	F: GCGGTGATGGTGAGTTCTTTCT R: ATCGAGAGGGAGGACCATTACAGT	qRT-PCR	
actin-related protein (housekpeeing)	F: ACTGTGAGGAGATGCAGAAACGCA R: GCTGTGTCACGGGCATTCAATGYT	qRT-PCR	

explant	callus ^ª	shoot ^ª	rootª
106	8	1	0
100	6	1	0
98	3	0	0
100	6	1	0
114	11	3	1
90	10	2	0
118	15	2	2
103	17	11	4 ^c
42	> 15	> 5	5
	explant 106 100 98 100 114 90 118 103 42	explant callus ^a 106 8 100 6 98 3 100 6 114 11 90 10 118 15 103 17 42 >15	explant callus ^a shoot ^a 106 8 1 100 6 1 98 3 0 100 6 1 98 3 0 100 6 1 114 11 3 90 10 2 118 15 2 103 17 11 42 >15 >5

Supplemental Table S2. Summary of transformation response from multiple trials

^aNumbers of putative independent events are shown.

^bOnly subsets of events were advanced through the regeneration process.

^cOne event was later confirmed to contain only the TUA1dEY transgene.

Wild type



Supplemental Fig. S1. Abnormal vascular development was observed in nonviable transgenic lines. A-C, Wild type; D-E, transformants derived from co-transformation of *TUA1* and *TUB15* (A1B15); F-H, transformants derived from co-transformation of *TUA5* and *TUB15* (A5B15). Scale bar = 1 cm (A, D, F), 500 µm (B) or 125 µm (C, E, G, H).

				A1dEYB15		A1dEY		A1dEYB9			
м	wild-type	A1dYB9	м _	#11	#12	#5	#17	#1	#2	N	н
A (HPT)		- 62 (-, 62	8					-	-	-	-
B (NPTII)								0	0	0	0
=		NUMBER OF									
=			-							-	
					- 24. (*						
C (TUA1d	Y/dEY transgene)										
= -											
						•					
D (<i>TUB9</i> t	ransgene)		5000 G			=		-	-	-	-
=											
E (TUB15	transgene)										
-			-								
F GT (hou	sekeeping)			-	-			-		~	-
			-								
0		Contraction of the second									

Supplemental Fig. S2. PCR analysis of wild-type and transgenic plants using gene-specific primers. (A) Selectable marker hygromycin phospho-transferase (*HPT*) gene from the *TUA* constructs. (B) Selectable marker neomycin phosphotransferase II (*NPTII*) gene from the *TUB* constructs. (C) *TUA1/dY/dEY* transgene. (D) *TUB9* transgene. (E) *TUB15* transgene. (F) A single-copy glycosyltransferase (*GT*) gene Potri.016G014500 as housekeeping control. A1dY, TUA1dY; A1dEY, TUA1dEY; B9, TUB9; B15, TUB15; M, molecular weight markers; N and H, vector controls with the NPTII or HPT construct, respectively. The A1dEY #5 and #17 lines was originally obtained from transformation with the A1dEYB15 combination, but found to harbor only the *TUA* and *HPT* transgenes.



Supplemental Fig. S3. Stem cross sections of wild-type and transgenic plants. Paraffin sections (10 μ m) were stained with toluidine blue and visualized under a light microscope. Scale bar = 125 μ m (A-E) or 500 μ m (F-J).



Supplemental Fig. S4. Total sugars and glycosyl composition of sequential cell wall extracts of wild-type and transgenic plants. (A) Total sugars in each cell wall fraction as measured by the phenol-sulfuric acid method. (B) Total sugar yield in each cell wall fraction recovered by methanolysis. (C-J) Sugar composition expressed as mol % (C-F) or as µg sugar/g cell wall material (G-J) in each fraction. Data are mean \pm SE of n=3-9 in (A) or n= 3-5 in (B-J). Refer to the right axis for Xyl in C, D, F, G, H and J. Statistical significance was determined by two-sample *t*-test (*** *P* ≤ 0.01; ** *P* <0.05; * *P* <0.1).



