Table S1. Gene-specific primers used for PCR amplification.

Name	Purpose	Forward primer (5'-3')	Reverse primer (5'-3')
FOX	FOX insertion gene cloning	TTTTACGACTCAATGACAAGA	CGATGGGGGTGTTCTGCTGGT
PtoLAC14	Gene cloning	CGGGATCCATGGAGTACGCTTGCTGGCTCC	CGGGATCCTCAACATTTTGGAAGGTCGCTT
	Cas9-Target1	GTCACCCTGCTTTGGTCCAGTGCA	AAACTGCACTGGACCAAAGCAGGG
	Cas9-Target2	GTCAGCGTTTAGGCAAGACAACCA	AAACTGGTTGTCTTGCCTAAACGC
	Cas9-Seq	GGCGATATGGAGTACGCTTG	ATTCAGCTAATACAACAACC
	Q-PCR	TGCCTCTTCCCTGCTTTGGTCC	GCCATTGACGGTAACAATCGGC
Hyg	PCR	ATCGGACGATTGCGTCGCATC	GTGTCACGTTGCAAGACCTG
PtoUBQ	Q-PCR	GTTGATTTTTGCTGGGAAGC	GATCTTGGCCTTCACGTTGT
ProAtLAC4	Promoter cloning	ACTGAGCTCACCATAGTTTTCATTGGAC	ATACCATGGCTCCCTCTCTATCTTTCTC
AtGAPDH	Q-PCR	GCAACATACGACGAAATCAAGAA	CGACACGAGAACTGTAACCCC

Table S2. Information of related genes.

No.	Genes	Accessions	Sequence identity to PtrLAC14	References
1	*PtrLAC2*	*Potri.001G184300.1*	61.24	Bryan *et al*., 2016
2	*PtrLAC3*	*Potri.001G206200.1*	67.26	Bryan *et al*., 2016
3	*PtrLAC14*	*Potri.006G096900.1*	100	Lu *et al*., 2013
4	*AtLac4*	*AT2G38080.1*	75.85	Berthet *et al*., 2011
5	*AtLac17*	*AT5G60020.1*	55.88	Berthet *et al*., 2011
6	*SofLAC*	*SCVPRZ3027A08.g*	57.54	Cesarino *et al*., 2013
7	*BdLAC5*	*Bradi1g66720.1*	56.99	Wang *et al*., 2015

Ptr: *P. trichocarpa*, At: *A. thaliana*, Sof: *Saccharum officinarum*, Bd: *Brachypodium distachyon*.

Table S3. Cell wall composition (% dry matter) of raw material.

Samples	Cellulose	Hemicelluloses	Lignin
WT1	36.73±0.91	22.16±0.08	23.70±0.83
OE-L1	36.62±0.55	22.30±0.03	26.52±0.87
OE-L2	36.95±0.59	22.30±0.31	26.32±0.76
OE-L5	37.07±1.14	22.33±0.41	27.35±0.66
WT2	37.79±0.69	22.30±0.25	22.39±0.24
KO-L1	36.73±0.91	22.33±0.41	20.88±0.23
KO-L3	36.90±0.99	23.30±0.31	20.72±0.11

Table S4. Lignin composition of raw material.

Samples	Lignin monolignols (μmol/g biomass)			Lignin monolignols (% total)		
	G	S	H	G	S	H
WT1	143.42±3	203.45±8	1.44±0.1	41.18	58.41	0.41
OE-L1	160.05±4	195.28±5	1.64± 0.1	44.84	54.71	0.46
OE-L2	165.01±5	203.48±8	2.46±0.2	44.48	54.86	0.66
OE-L5	165.27±7	198.55±6	1.81±0.1	45.20	54.30	0.50
WT2	172.24±6	206.20±4	2.40± 0.2	45.23	54.14	0.63
KO-L1	154.64±5	207.97±5	2.41±0.2	42.36	56.97	0.66
KO-L3	155.20±5	210.82±6	2.71±0.3	42.09	57.17	0.74

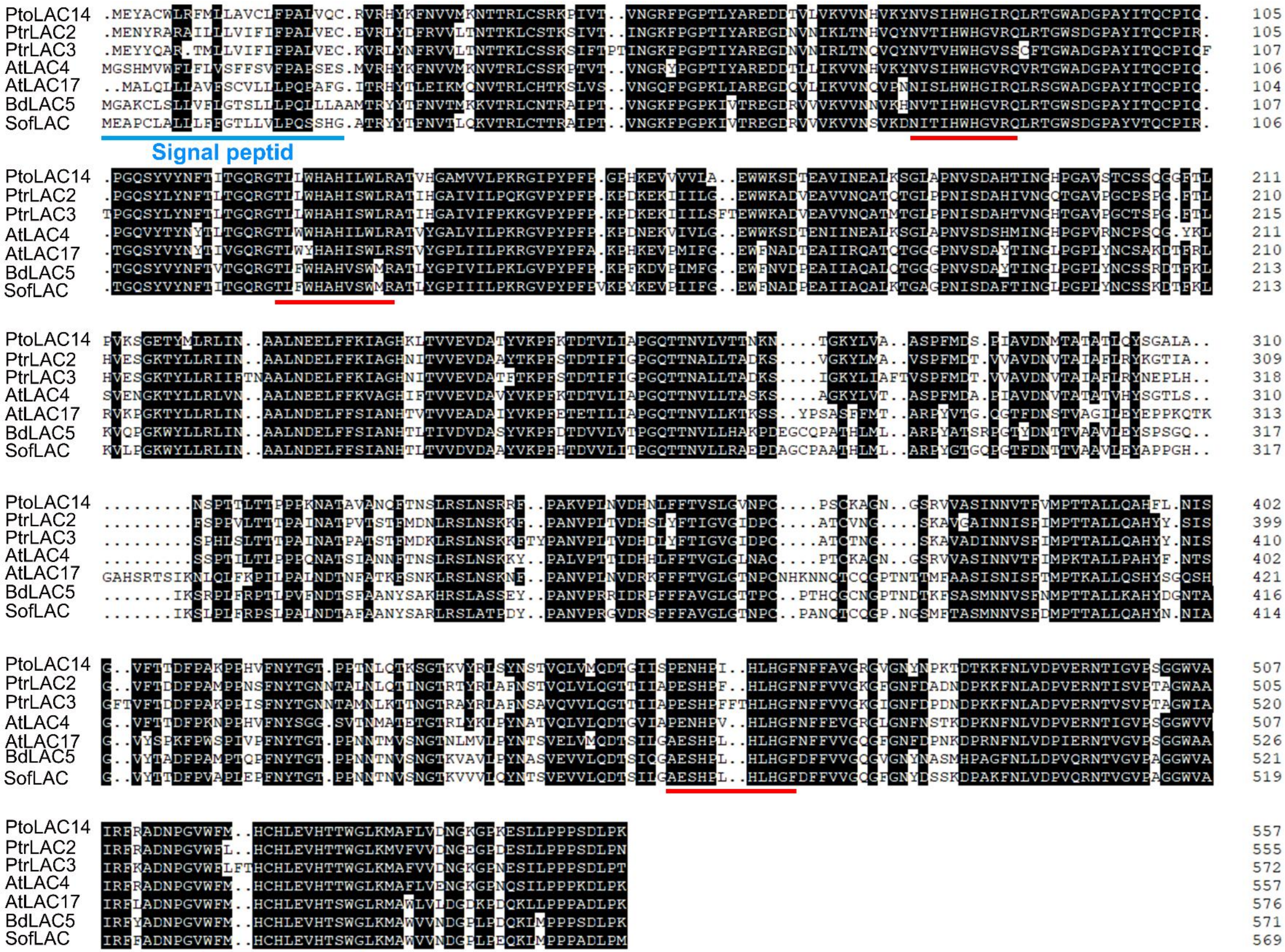


Fig. S1 Multiple alignment of *PtoLAC14* with other plant laccase proteins known to be involved in lignification. Ptr: *P. trichocarpa*, At: *A. thaliana*, Sof: *Saccharum officinarum*, Bd: *Brachypodium distachyon.*

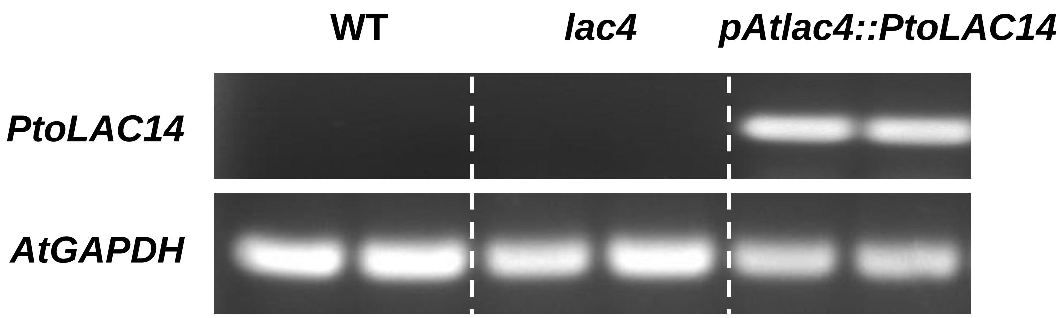


Fig. S2 Expression of *PtoLAC14*.

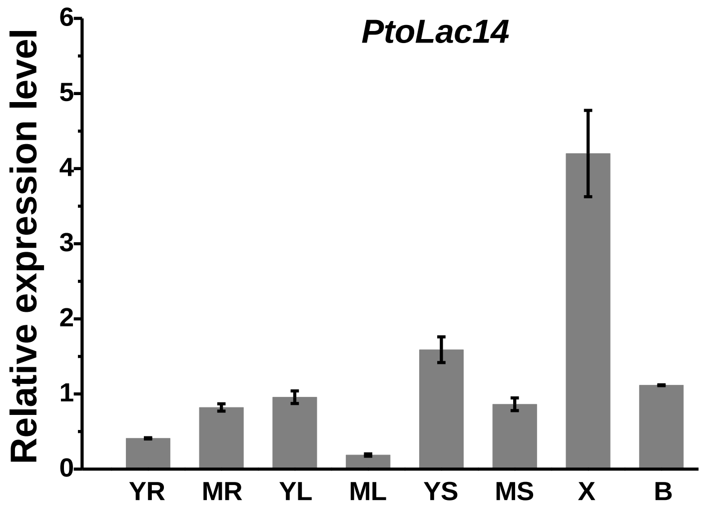


Fig. S3 The expression profiling of *PtoLAC14* by Q-PCR analysis.
YR, young root; MR, mature root; YL, young leaf; YL, ML, mature leaf; YS, young stem; MS, mature stem; X, xylem; B, bark.

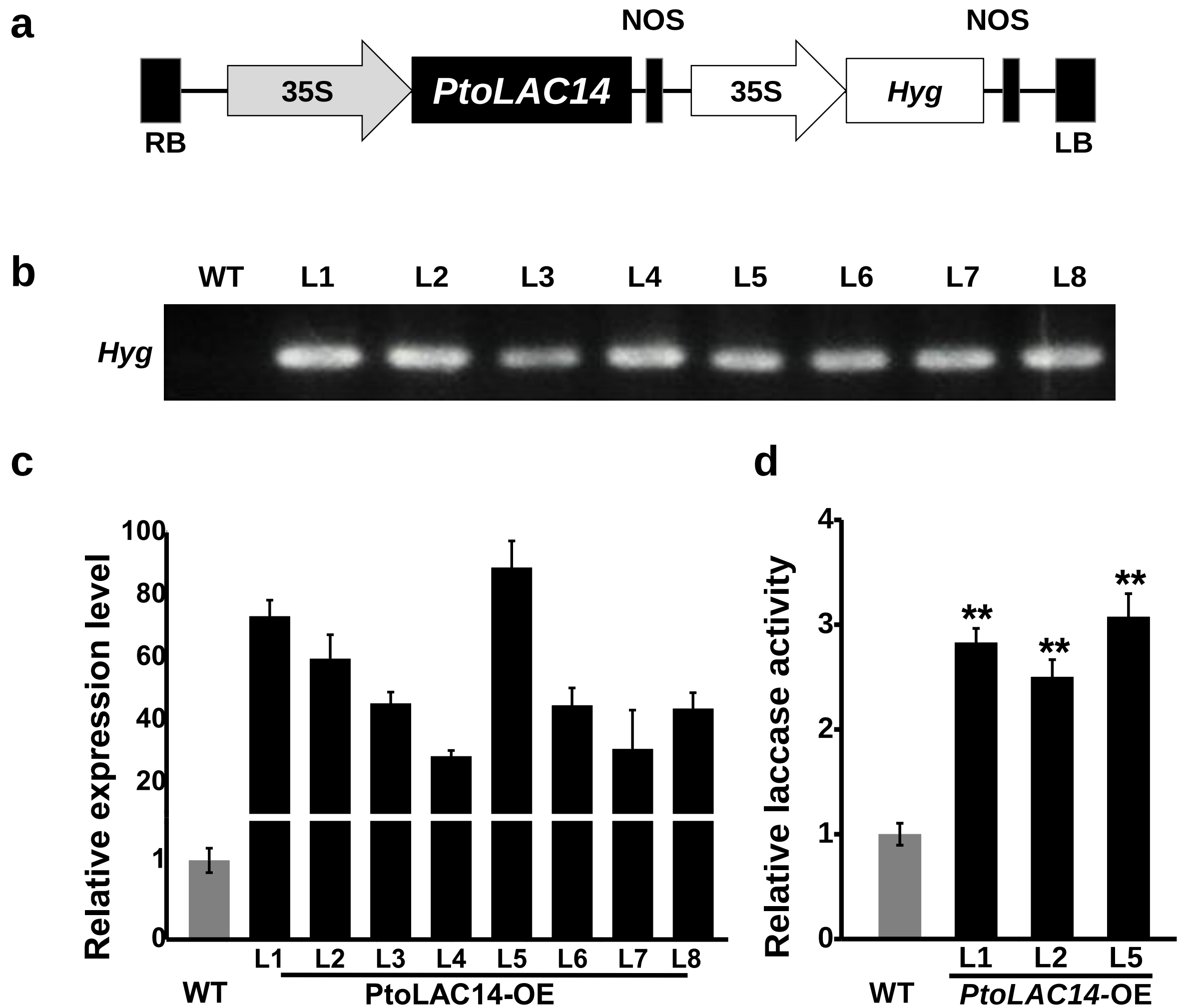
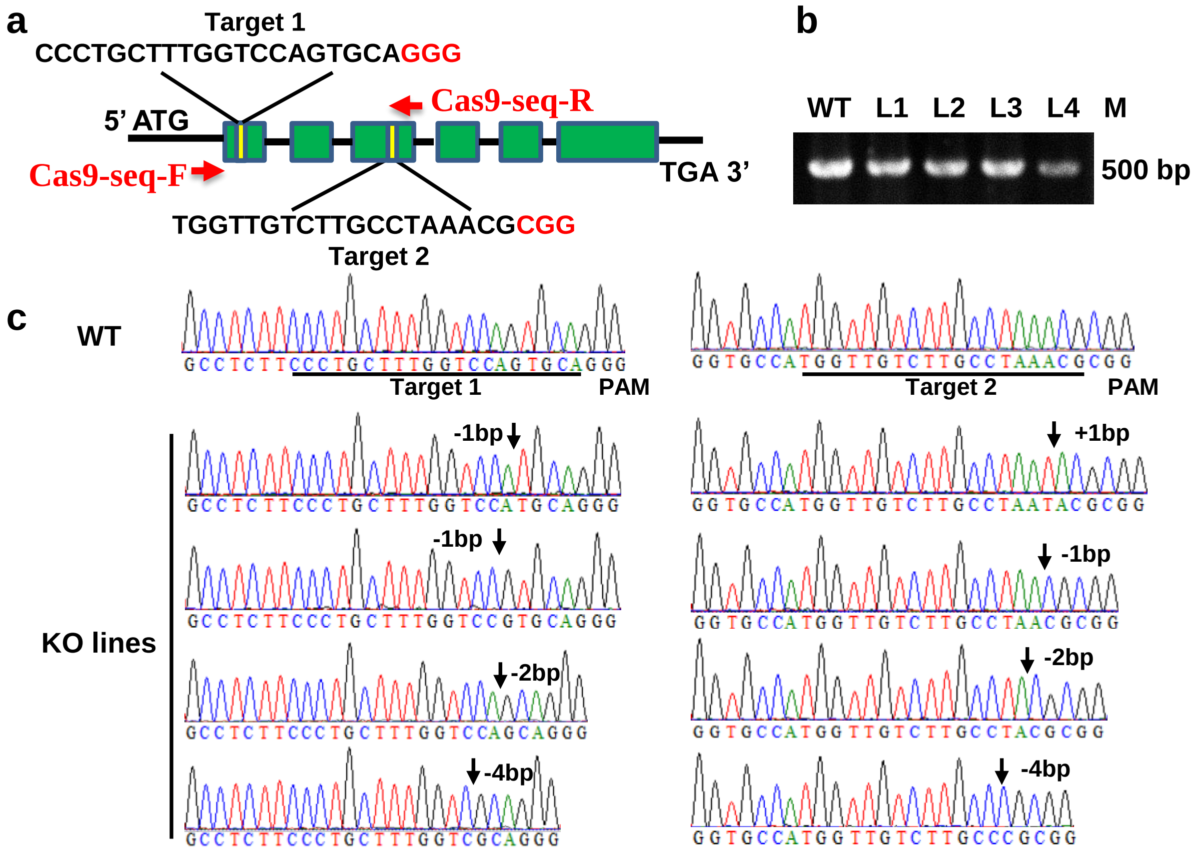


Fig. S4 Generation of PtoLAC14-OE transgenic poplars. (a) Diagram of the PtoLAC14-OE vector. (b) The Hyg levels in the PtoLAC14-OE lines. (c) The expression levels of PtoLAC14 in the PtoLAC14-OE lines. The poplar ubiquitin gene was used as an internal control. (d) Quantification of laccase activity with ABTS as the substrate. Data represent mean ± SD of three biological replicates. Statistical analyses were performed using Student's *t* test as $^{**}P < 0.01$.



Target gene	No. of clones examined	No. of clones with mutations	Mutation rate (%)
PtoLAC14	50	42	84

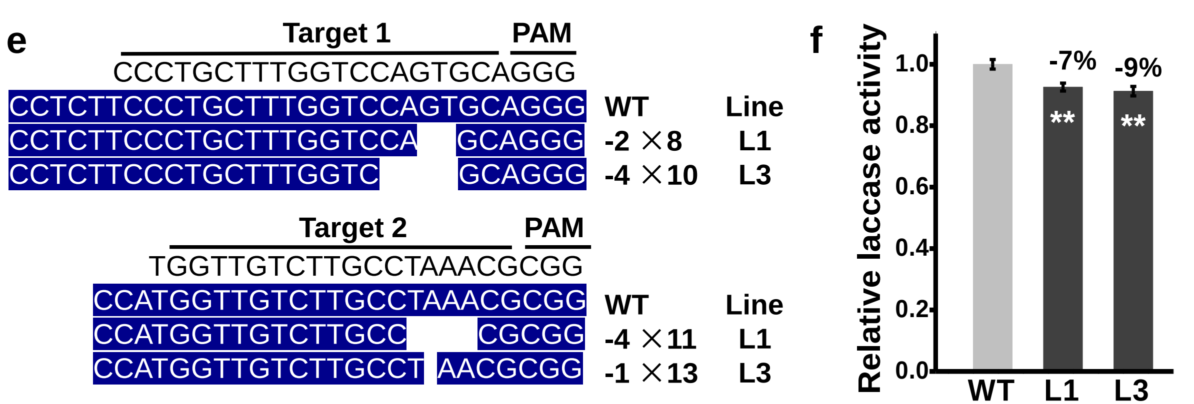


Fig. S5 Collection of *PtoLAC14*-KO transgenic poplars.

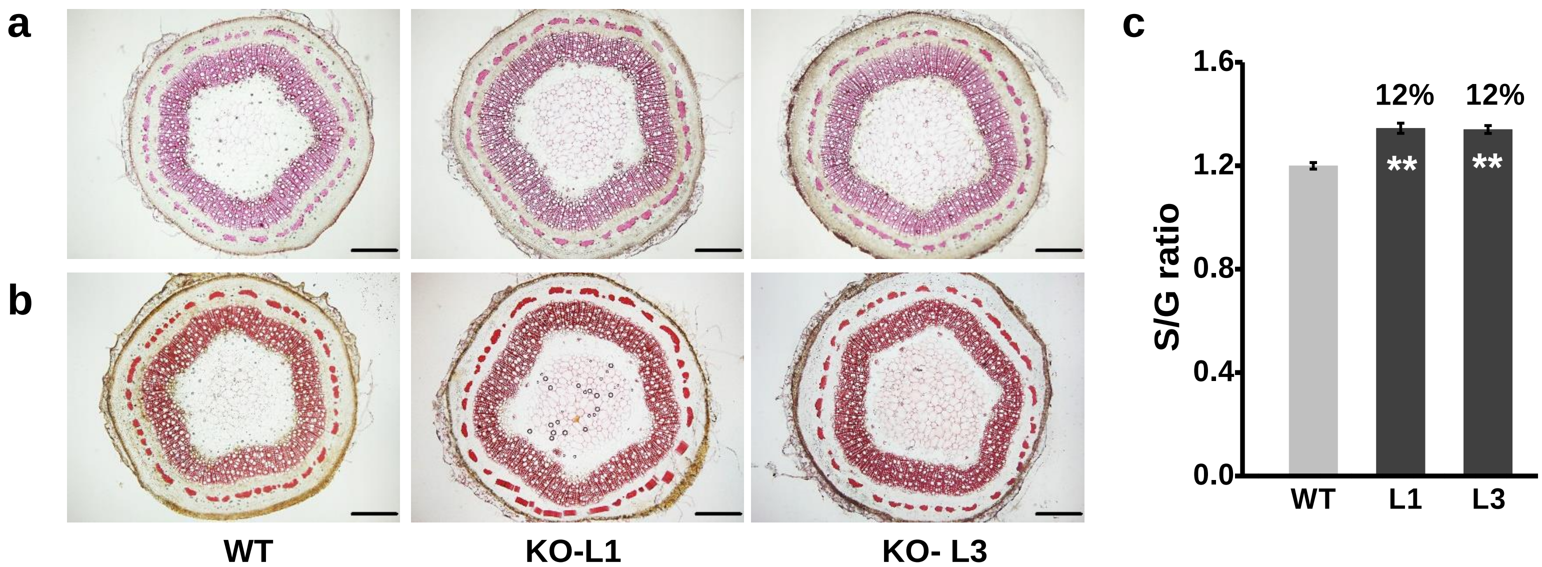


Fig. S6 Lignin and monomer lignin in *PtoLAC14*-KO transgenic poplar lines. (a) Phloroglucinol–HCl staining of stem cross sections from WT and *PtoLAC14*-KO lines. Scale bars as 100 μm. (b) Histochemical stained with Mäule reagent for lignin monomer composition of stem cross sections from WT and *PtoLAC14*-KO lines. Scale bars as 100 μm. (c) The monomer lignin S/G ratio in WT and *PtoLAC14*-KO lines. All data as means ± SD (n = 3). Student's *t*-test was performed between the transgenic line and WT as $**P < 0.01$.