

Molecular cloning and characterization of *PtrLAR3*, a gene encoding leucoanthocyanidin reductase from *Populus trichocarpa* and its constitutive expression enhances fungal resistance in transgenic plants

Authors:

¹Li Yuan, ¹Lijun Wang, ²Zujing Han, ¹Yuanzhong Jiang, ²Lili Zhao, ¹Hong Liu, ¹Li Yang, ¹Keming Luo*

¹Key Laboratory of Eco-environments of Three Gorges Reservoir Region, Ministry of Education, Institute of Resources Botany, School of Life Sciences, Southwest University, Chongqing 400715, China

²Beijing Genomics Institute-Shenzhen, Shenzhen 518083, China

Supplementary Material

Supplementary Table S1: Comparison of changes in expression levels of PA biosynthetic genes leading to PA accumulation following *Marssonina brunnea* f.sp. *multigermtubi* infection using digital gene expression (DGE) analysis.

Gene ID	Gene_length	Read count (WT)	Read count (<i>M.brunnea</i> infection)	WT4-RPKM	<i>M.brunnea</i> infection-RPKM	Log2 Ratio (<i>M.brunnea</i> infection/WT)	Up-/down-regulation (<i>M.brunnea</i> infection/WT)	P-value
POPTR_0006s12870	2145	5328	4264	669.5861187	589.910896	-0.182772563	Down	6.54E-10
POPTR_0008s03810	2136	306	439	38.61799306	60.99016248	0.659303369	Up	5.95E-10
POPTR_0006s12870	2145	2526	1262	356.5261046	589.910896	-0.182772563	Down	6.54E-10
POPTR_0016s09230	2148	756	838	94.87614725	115.7727262	0.287178091	Up	7.13E-05
POPTR_0010s23100	2136	14	22	1.766836284	3.056454612	0.790690778	Up	0.10728
POPTR_0019s15110	1518	583	321	103.5300217	62.75231894	-0.722308504	Down	2.17E-13
POPTR_0013s15380	1518	735	541	130.5224116	105.7601388	-0.303501574	Down	0.000194042
POPTR_0014s14200	1191	201	170	45.49396801	42.35782759	-0.103046673	Down	0.495438
POPTR_0001s14320	1176	1	47	0.229225116	11.8600646	5.693202933	Up	0.000567202
POPTR_0001s14310	1173	0	72	0.001	18.21507663	14.15284544	Up	0
POPTR_0003s17530	1176	5	64	1.146125578	16.1498752	3.816685987	Up	3.48E-05
POPTR_0002s03500	1041	29	272	7.50959975	77.53801754	3.368095928	Up	3.81E-11
POPTR_0006s08750	987	13	143	3.550550725	42.99474755	3.5980457	Up	4.76E-07
POPTR_0004s03030	1008	32	232	8.557737646	68.30051388	2.996595077	Up	6.67E-12
POPTR_0003s11900	1086	0	20	0.001	5.465082078	12.41602745	Up	3.39E-07
POPTR_0001s08410	1083	5	177	1.244546334	48.49995417	5.284291537	Up	3.48E-05
POPTR_0008s11540	1059	25	118	6.363756748	33.06606885	2.377400941	Up	4.11E-10
POPTR_0015s03360	1050	0	13	0.001	3.674096609	11.84317385	Up	6.13E-05
POPTR_0013s07050	1566	95	368	16.35314809	69.73528488	2.092320429	Up	0
POPTR_0010s21980	672	95	109	38.10867545	48.13419836	0.336942798	Up	0.095666

^aThe gene expression level is calculated by using RPKM method (Reads Per kb per Million reads) (Mortazavi et al., 2008), and the formula is shown as follows: $RPKM = 10^6 C/NL/10^3$. Given RPKM(A) to be the expression of gene A, C to be number of reads that uniquely aligned to gene A, N to be total number of reads that uniquely aligned to all genes, and L to be number of bases on gene A.

A Student's *t*-test (two-sample, unpaired, one-sided) was performed to test significance (*P*) of up- or downregulation of each transcript between infected and control leaves.

Supplementary Figure S1: Photographs of *PtrLAR3* overexpressing *Populus tomentosa* Carr. after four weeks of growth. No phenotypic or growth differences were observed between *PtrLAR3* overexpressing and control plants under the same growth conditions.

Supplementary Figure S2: PCR analysis of transgenic poplar plants. Genomic DNAs were isolated from hygromycin-resistant plants transformed with the *35S:PtrLAR3* vector. PCR amplification using primers specific for the production of a 562-bp *PtrLAR3* fragment. M, D2000 DNA Ladder; WT, non-transgenic plants; P, corresponding plasmid DNA (positive control); Lanes 1–10, independent transgenic lines. Numbers on the left indicate DNA marker sizes in base pairs.

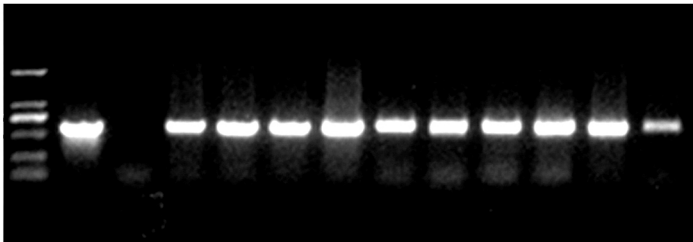
Reference

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. 2008 Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods* 5, 621-628.



M P WT 1 2 3 4 5 6 7 8 9 10

750 bp
500 bp



562 bp