



Supplementary Materials: Wood Architecture and Composition Are Deeply Remodeled in Frost Sensitive *Eucalyptus* Overexpressing CBF/DREB1 Transcription Factors

Phi Bang Cao, Raphaël Ployet, Chien Nguyen, Annabelle Dupas, Nathalie Ladouce, Yves Martinez, Jacqueline Grima-Pettenati, Christiane Marque, Fabien Mounet and Chantal Teulière



Table S1. Expression of genes related to monolignol biosynthesis pathway in CBF-OE transgenic *Eucalyptus*.

Gene Family (<i>Bona Fide</i> Analysed/Total)	Gene Alias (* <i>Bona Fide</i>)	Gene Accession	Student's T test Performed	EguCBF_K-OE	Significant (T test <i>P</i> value)	EguCBF_Q-OE	Significant (T test <i>P</i> value)	Cold Responsive Ployet et al., 2017
PAL (2/2)	EgrPAL1	Eucgr.A01144	+	2.97		4.78	<i>P</i> < 0.05	+
	EgrPAL2	Eucgr.C03570	+	1.77	<i>P</i> < 0.05	1.53		
	*EgrPAL3	Eucgr.G02848	+	1.22		0.59		+
	EgrPAL4	Eucgr.G02849	+	1.1		0.86		
	EgrPAL5	Eucgr.G02850	+	2.21	<i>P</i> < 0.05	2.55	<i>P</i> < 0.05	
	EgrPAL7	Eucgr.G02852	+	1.1		0.83		
	EgrPAL8	Eucgr.J00907	+	0.81		0.87		+
	*EgrPAL9	Eucgr.J01079	+	2.09	<i>P</i> < 0.05	1.49		+
	C4H (2/2)	*EgrC4H2	Eucgr.C00065		0.58		2.79	
*EgrC4H1		Eucgr.J01844		0.75		3.4		+
4CL (1/1)	*Egr4CL1	Eucgr.C02284	+	1.02		1.18		+
HCT (2/2)	EgrHCT1	Eucgr.F03972		2.6		2.38		+
	*EgrHCT4	Eucgr.F03978		1.94		2.9		
	*EgrHCT5	Eucgr.J03126	+	1.14		0.67		+
C3H (2/2)	EgrC3H1	Eucgr.A02185	+	0.85		0.43		+
	EgrC3H2	Eucgr.A02188	+	2.66	<i>P</i> < 0.05	2.45		+
	*EgrC3H3	Eucgr.A02190		2.53		3.2		+
	*EgrC3H4	Eucgr.G03199	+	0.64		2.99	<i>P</i> < 0.05	+
CCoAOMT (2/2)	*EgrCCoAOMT1	Eucgr.I01134		0.9		3.07		+
	*EgrCCoAOMT2	Eucgr.G01417		0.96		2.2		+
CCR (1/1)	*EgrCCR1	Eucgr.J03114	+	0.87		0.79		+
	EgrCCR2	Eucgr.F03954	+	3.46	<i>P</i> < 0.05	7.13	<i>P</i> < 0.05	+
F5H (1/1)	EgrF5H2	Eucgr.I02371	+	1.45		1.12		
	*EgrF5H1	Eucgr.J02393		1		2.77		

COMT (1/1)	*EgrCOMT1	Eucgr.A01397	+	0.93	0.61	
	EgrCOMT2	Eucgr.A01389		1.95	3.7	+
	EgrCOMT3	Eucgr.A01392		0.56	2	+
	EgrCOMT6	Eucgr.K00041		2.38	3.82	+
CAD (1/2)	*EgrCAD2	Eucgr.G01350	+	1.22	1	+

Expression values are ratios of average transcript level detected in 3 biological replicates per line, relative to control transgenic plants. Genes short names are according to [32]: PAL, phenylalanine ammonialyase; C4H, Cinnamate-4-hydroxylase; 4CL, 4-hydroxycinnamate CoA ligase; HCT, hydroxycinnamoyl transferase; C3H, p-coumarate 3-hydroxylase; CCR, cinnamoyl CoA reductase; CCoAOMT, caffeoyl-CoA 3-O-methyltransferase; F5H, ferulate 5-hydroxylase; COMT, caffeic acid O-methyltransferase; CAD, cinnamyl alcohol dehydrogenase. In bold and identified with (*) are the *bona fide* genes belonging to the lignin toolbox in *Eucalyptus* [32]. Ratios higher than 1.2 are highlighted in bold. Statistical significance was assessed for 18 genes using Student's T test on 3 to 6 biological replicates.



Table S2. Composition of MS medium (W4) promoting shoot elongation.

Macroelements (mg. L⁻¹)		Fe-EDTA (mg. L⁻¹)	
NH ₄ NO ₃	1650	FeSO ₄ . 7H ₂ O	27.85
KNO ₃	1900	Na ₂ EDTA	37.25
CaCl ₂ .2H ₂ O	440	Vitamins (mg.L⁻¹)	
MgSO ₄ . 7H ₂ O	370	Nicotinic acid	0.1
KH ₂ PO ₄	170	Thiamine-HCl	0.5
Oligoelements (mg.L⁻¹)		Pyridoxine-HCl	0.1
MnSO ₄ . H ₂ O	16.9	Myo-inositol	100
ZnSO ₄ . 7H ₂ O	10.6	Saccharose (g. L⁻¹)	
H ₃ BO ₃	6.2	Phytigel (g. L ⁻¹)	3.5
KI	0.83	Hormones (μM)	
Na ₂ MoO ₄ . 2H ₂ O	0.25	BAP*	0.5
CuSO ₄ . 5H ₂ O	0.025		
CoCl ₂ .6H ₂ O	0.025	pH	
			6

(*) Added after sterilization.

Table S3. List of oligonucleotide sequences used as primers for RT-qPCR reactions.

Gene Name	Accession Number	Forward Primers	Reverse Primers
Transgenes			
<i>EguCBF_K-OE</i>		GTACAAAAAAGCAGGCTATATGAACC C	TGGCACTCCTTCCGTCAGAT
<i>EguCBF_Q-OE</i>		CCAGAGAGGACAACGGGAAC	GAAAGCTGGGTAACATGGAATAGCTC
Genes Involved in Lignin Biosynthesis			
<i>EgrPAL1</i>	Eucgr.A01144	TCAAGGATTGCGGGTCGTATCC	ACCCTTTCTCCGGTCAGCAAAC
<i>EgrPAL2</i>	Eucgr.C03570	GATGGCTTGCTGCTTGAATCGC	AGACGGGCTCATGTTCAACGAC
<i>EgrPAL3</i>	Eucgr.G02848	TGGGTGATGGAGAGCATGAGCAAG	ATGCCGGCGTTCAAGAACCTTATG
<i>EgrPAL4</i>	Eucgr.G02849	CAGACGGAGCTCATAAGGTTCTTG	GTGTTGATCCTCACGAGCATGG
<i>EgrPAL5</i>	Eucgr.G02850	GCGCATGTGAATTTCTTCATAGC	ATCGAGCGAGGATCAAGGTGAG
<i>EgrPAL7</i>	Eucgr.G02852	GGCATATTCGGCAATGGGACTG	TGTTGATCCTCACGAGCATTGCG
<i>EgrPAL8</i>	Eucgr.J00907	GGAGTGGATCATGGAGAGCATGTG	ACACCGGCGTTCAAGAATCTGATG
<i>EgrPAL9</i>	Eucgr.J01079	TGGAAGGAGCCAGAATCATGCC	TTGTGGACCGACCATAATGTGC
<i>EgrC4H1</i>	Eucgr.J01844	TGAGAAGGGTGGCCAATTCAGC	TGGCTTCAAGACGATGGTGGAG
<i>EgrC4H2</i>	Eucgr.C00065	ACTGTCGCTTTGAGCCCGTTG	GGCTGCCCATTTGGTTTAAATGCG
<i>Egr4CL1</i>	Eucgr.C02284	AGGAACGCGGAGATGAAGATCG	CGGCGTCGTTTCAAGATAAACCTTTC
<i>EgrHCT1</i>	Eucgr.F03972	CCATTGAAGCTCGCAGTCTAGC	GATATCATCCGAGTGCCTTATGC
<i>EgrHCT4</i>	Eucgr.F03978	GCGCATGTTGTTTATTCTTCATGC	GCAATGCATCCAATCCCCTACTGC
<i>EgrHCT5</i>	Eucgr.J03126	CGGAAGCTCATTCCAGCTGTAGAC	TTGAAGTACGTCACCTGCAACAC
<i>EgrC3H1</i>	Eucgr.A02185	CGGCAACCTCTACGACATCAAATC	AGCTTGACACGACCACGTTTCCAG
<i>EgrC3H2</i>	Eucgr.A02188	ACGTGAAGGTCAGGAAGGTGTG	GCACGGTCTTCTCGAATTATCGG
<i>EgrC3H3</i>	Eucgr.A02190	TGCACCAACCCTGATAATTCGG	CGCCAGCCTCGTTATGTTGTTG
<i>EgrC3H4</i>	Eucgr.G03199	GGAAGTTATGACCTGAGCGAGGAC	GCCAGTGATCATGTCCCAAAGG
<i>EgrCCoAOMT1</i>	Eucgr.I01134	AGAAGGCCCTGCTTTGCCTATTC	AACGACCCTTGCTTCCCCTCTTC
<i>EgrCCoAOMT2</i>	Eucgr.G01417	ATCAGCTCGTGCAAGATGAGAAG	TGTAGTTGTCCTTGTGCGGCATCC
<i>EgrCCR1</i>	Eucgr.J03114	GCGATGTGGTGGAAATCCTTGC	GGTTTACCTCATCAGAGCACTTG
<i>EgrCCR2</i>	Eucgr.F03954	AGAGGTTGACTCTGTGCAAGGC	GCTGGCTCCAACATTTGCTCTC
<i>EgrF5H1</i>	Eucgr.J02393	AAGCAAATGGAGGGTCGGGTTG	TCCAAATCTTGACGCCCTCCTG
<i>EgrF5H2</i>	Eucgr.I02371	ATTGAGACGAGGCATCGAACCG	CGCATGCAAAGCCAGCCATTTCC
<i>EgrCOMT1</i>	Eucgr.A01397	CACGTGATTGAAGACGCTCCAC	CTCCAGTCATGGCATATCCACTTC
<i>EgrCOMT2</i>	Eucgr.A01389	ACAGCTTGAAGTGTGCTCTCCTG	AAGGCTCATTTGCGACCTATCG
<i>EgrCOMT3</i>	Eucgr.A01392	AGACGATGGTTGATGTTGGAGGTG	AACGTGTTCCACACCGGGATAG
<i>EgrCOMT6</i>	Eucgr.K00041	CTGATCATGATGGCGCACAACC	CAAAGGCAAACGCACGCGATATG
<i>EgrCAD2</i>	Eucgr.G01350	AGTCCCCTTAGGTTAGCTGTC	AAAGAGAAATCCACTTCCGCTGTC
Reference Genes			
<i>EgrSAND</i>	Eucgr.B02502	TTGATCCACTTGCGGACAAGGC	TCACCCATTGACATACACGATTGC
<i>EgrEF-1a</i>	Eucgr.B02473	ATGCGTCAGACTGTGGCTGTTG	TTGGTCACCTTGGCTCCACTTG
<i>EgrIDH</i>	Eucgr.F02901	AATCGACCTGCTTCGACCCTTC	TCGACCTTGATCTTCTCGAAACCC
<i>EgrPP2A1</i>	Eucgr.B03386	TCGAGCTTTGGACCGCATAACAAG	ACCACAAGAGGTACACATTGGC
<i>EgrPP2A3</i>	Eucgr.B03031	CAGCGCAAACAACCTGAAGCG	ATTATGTGCTGCATTGCCAGTC