

Supplementary Materials: Screening and Validation of Housekeeping Genes of the Root and Cotyledon of *Cunninghamia lanceolata* under Abiotic Stresses by Using Quantitative Real-Time PCR

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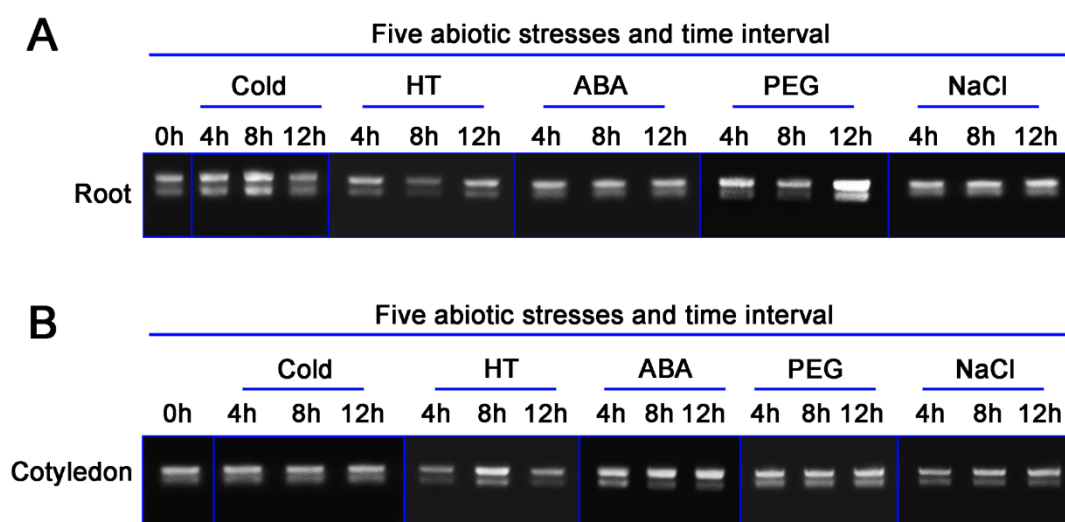


Figure S1. RNA gel electrophoresis of root (A) and cotyledon (B) sample sets.

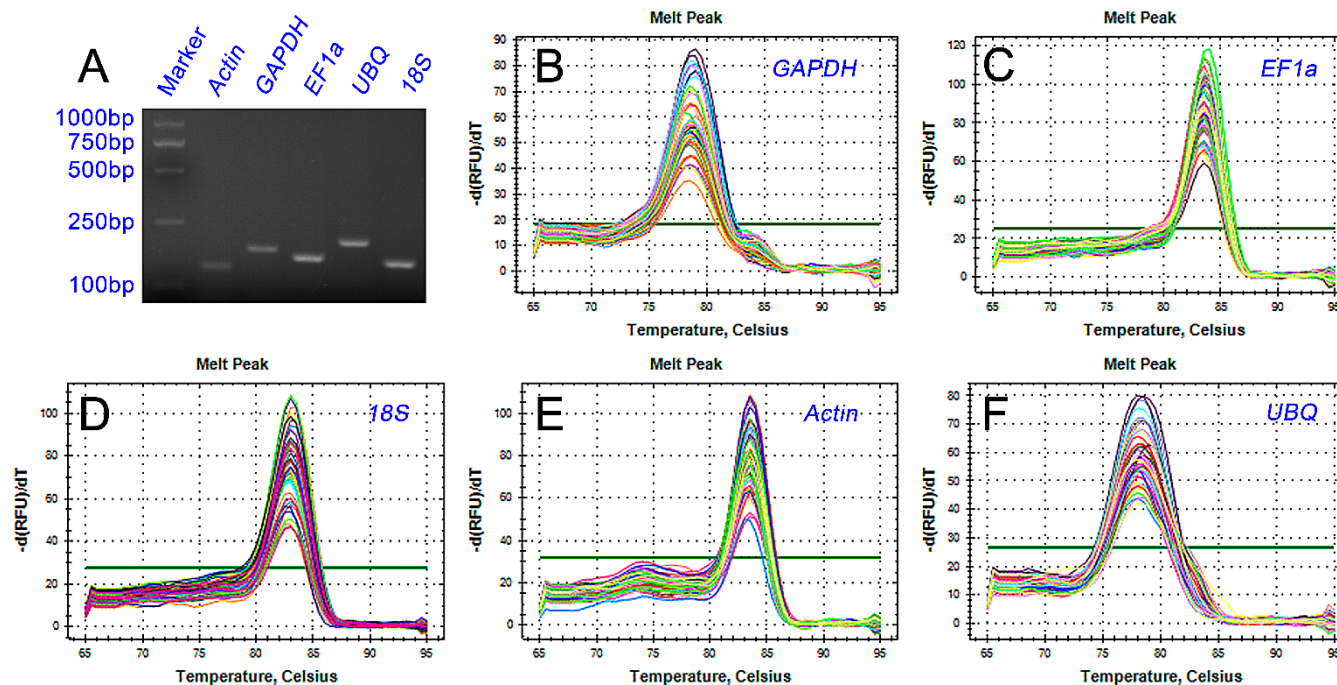


Figure S2. qRT-PCR amplification specificity and melt curve. Agarose gel electrophoresis of each HKG candidate gene (A); Melt curve of *GAPDH* (B); *EF1a* (C); *18S* (D); *Actin* (E) and *UBQ* (F). Gene fragments generated from qRT-PCR were isolated by 2.5% agarose gel electrophoresis. HT: High Temperature treatment; Cold: Cold treatment; ABA: Absciscic Acid treatment; NaCl: Sodium Chloride treatment; PEG: Polyethylene Glycol treatment.

Table S1. The transcriptome ID, *Arabidopsis* orthologous locus, primer sequences, amplicons length and PCR efficiencies for five candidate HKGs in Chinese fir.

Gene Abbreviation	Chinese Fir Transcriptome ID	Arabidopsis Orthologous locus	Primer Sequences (Forward/Reverse)	Amplicon Length (bp)	PCR Efficiency
<i>Actin</i>	comp117588_c0	AT3G18780	AAGCTCTCCTTGTGCTGTT/GACTTCTGGGCATCTGAATCT	138	2.05
<i>GAPDH</i>	comp51461_c0	AT1G79530	GCACCTATGTTTGTGGTTGGAGTA/ACCGTCTTCTGTGTAGCTGTTGTT	182	2.08
<i>EF1α</i>	comp126172_c0	AT5G60390	TGGCAAGGAGCTTGAGAAAGAACCCA/ACCCCAACAGCAACAGTCTGACGCAT	162	1.92
<i>UBQ</i>	comp129739_c0	AT1G65350.1	AATAAATGCTTCAAATGTCAGGCTA/TGAGATGGTCTGGTGTATGTCGTGG	207	2.03
<i>18S</i>	comp134344_c0	AT3G57000	GGGAACATTATCACGGACAGCATCAAC/TGCGACTAATGGTCCAGATAGACTCCT	154	2.01

Table S2. Specific data of CV and r^2 (root).

HT-Root				Cold-Root				PEG-Root			
Gene	CV	Gene	r^2	Gene	CV	Gene	r^2	Gene	CV	Gene	r^2
<i>UBQ</i>	1.601	<i>EF1a</i>	0.694	<i>Actin</i>	0.271	<i>GAPDH</i>	0.13	<i>Actin</i>	0.995	<i>UBQ</i>	0.033
<i>18s</i>	1.618	<i>Actin</i>	0.884	<i>18s</i>	1.31	<i>18s</i>	0.144	<i>UBQ</i>	1.086	<i>GAPDH</i>	0.094
<i>Actin</i>	2.315	<i>UBQ</i>	0.992	<i>UBQ</i>	4.071	<i>UBQ</i>	0.326	<i>GAPDH</i>	2.344	<i>18s</i>	0.281
<i>EF1a</i>	5.188	<i>18s</i>	0.996	<i>GAPDH</i>	5.168	<i>Actin</i>	0.721	<i>18s</i>	2.75	<i>Actin</i>	0.399
<i>GAPDH</i>	14.128	<i>GAPDH</i>	0.98	<i>EF1a</i>	9.494	<i>EF1a</i>	1	<i>EF1a</i>	3.427	<i>EF1a</i>	0.764
ABA-Root				NaCl-Root				Total-Root			
Gene	CV	Gene	r^2	Gene	CV	Gene	r^2	Gene	CV	Gene	r^2
<i>GAPDH</i>	0.684	<i>Actin</i>	0.293	<i>Actin</i>	0.098	<i>GAPDH</i>	0.009	<i>Actin</i>	1.593	<i>UBQ</i>	0.002
<i>Actin</i>	0.747	<i>GAPDH</i>	0.719	<i>18s</i>	1.375	<i>EF1a</i>	0.162	<i>18s</i>	2.384	<i>18s</i>	0.189
<i>UBQ</i>	1.537	<i>18s</i>	0.792	<i>EF1a</i>	3.426	<i>Actin</i>	0.274	<i>UBQ</i>	2.717	<i>GAPDH</i>	0.359
<i>18s</i>	2.362	<i>UBQ</i>	0.863	<i>UBQ</i>	3.889	<i>18s</i>	0.848	<i>EF1a</i>	5.847	<i>Actin</i>	0.387
<i>EF1a</i>	2.767	<i>EF1a</i>	0.931	<i>GAPDH</i>	4.083	<i>UBQ</i>	0.922	<i>GAPDH</i>	6.208	<i>EF1a</i>	0.425

Abbreviation: HT: High Temperature treatment; Cold: Cold treatment; ABA: Absciscic Acid treatment; NaCl: Sodium Chloride treatment; PEG: Polyethylene Glycol treatment; CV: Coefficient of Variance; r^2 : Coefficient of Determination.

Table S3. Specific data of CV and r^2 (cotyledon).

HT-Cotyledon				Cold-Cotyledon				PEG-Cotyledon			
Gene	CV	Gene	r^2	Gene	CV	Gene	r^2	Gene	CV	Gene	r^2
<i>GAPDH</i>	0.193	<i>GAPDH</i>	0.731	<i>GAPDH</i>	0.569	<i>Actin</i>	0.001	<i>18S</i>	1.273	<i>EF1a</i>	0.388
<i>18S</i>	0.244	<i>EF1a</i>	0.824	<i>UBQ</i>	0.674	<i>GAPDH</i>	0.026	<i>EF1a</i>	1.759	<i>18S</i>	0.545
<i>EF1a</i>	1.013	<i>UBQ</i>	0.867	<i>Actin</i>	1.617	<i>18S</i>	0.408	<i>GAPDH</i>	2.022	<i>UBQ</i>	0.638
<i>UBQ</i>	1.692	<i>18S</i>	0.966	<i>18S</i>	2.419	<i>UBQ</i>	0.726	<i>UBQ</i>	2.941	<i>Actin</i>	0.79
<i>Actin</i>	2.484	<i>Actin</i>	0.984	<i>EF1a</i>	2.874	<i>EF1a</i>	2.874	<i>Actin</i>	3.917	<i>GAPDH</i>	0.968
ABA-Cotyledon				NaCl-Cotyledon				Total-Cotyledon			
Gene	CV	Gene	r^2	Gene	CV	Gene	r^2	Gene	CV	Gene	r^2
<i>18S</i>	2.059	<i>18S</i>	0.806	<i>18S</i>	1.061	<i>EF1a</i>	0.529	<i>18S</i>	2.42	<i>Actin</i>	0.289
<i>UBQ</i>	2.343	<i>Actin</i>	0.824	<i>GAPDH</i>	2.908	<i>UBQ</i>	0.551	<i>Actin</i>	3.266	<i>18S</i>	0.419
<i>Actin</i>	2.502	<i>UBQ</i>	0.874	<i>UBQ</i>	4.024	<i>Actin</i>	0.78	<i>UBQ</i>	3.377	<i>EF1a</i>	0.692
<i>EF1a</i>	3.143	<i>GAPDH</i>	0.901	<i>Actin</i>	4.131	<i>GAPDH</i>	0.949	<i>GAPDH</i>	3.533	<i>UBQ</i>	0.767
<i>GAPDH</i>	3.651	<i>EF1a</i>	0.962	<i>EF1a</i>	5.384	<i>18S</i>	1	<i>EF1a</i>	3.887	<i>GAPDH</i>	0.794

Abbreviation: HT: High Temperature treatment; Cold: Cold treatment; ABA: Absciscic Acid treatment; NaCl: Sodium Chloride treatment; PEG: Polyethylene Glycol treatment; CV: Coefficient of Variance; r^2 : Coefficient of Determination.

Table S4. Summary of results generated from different algorithms (root).

Different Algorithms	Tissue Under the Different Treatments					
	HT-Root	Cold-Root	PEG-Root	ABA-Root	NaCl-Root	Total-Root
NormFinder (Stab.)	<i>Actin</i>	<i>Actin</i>	<i>UBQ</i>	<i>GAPDH</i>	<i>Actin</i>	<i>Actin</i>
BestKeeper (CV)	<i>UBQ</i>	<i>Actin</i>	<i>Actin</i>	<i>GAPDH</i>	<i>Actin</i>	<i>Actin</i>
BestKeeper (r^2)	<i>GAPDH</i>	<i>EF1a</i>	<i>EF1a</i>	<i>EF1a</i>	<i>UBQ</i>	<i>EF1a</i>
geNorm (M value)	<i>18S</i>	<i>18S</i>	<i>Actin</i>	<i>Actin</i>	<i>18S</i>	<i>18S</i>
RankAggreg	<i>GAPDH</i>	<i>Actin</i>	<i>EF1a</i>	<i>GAPDH</i>	<i>Actin</i>	<i>Actin</i>

Abbreviation: HT: High Temperature treatment; Cold: Cold treatment; ABA: Absciscic Acid treatment; NaCl: Sodium Chloride treatment; PEG: Polyethylene Glycol treatment; Stab. : Stability; CV: Coefficient of Variance; r^2 : Coefficient of Determination.

Table S5. Summary of results generated from different algorithms (cotyledon).

Different Algorithms	Tissue Under the Different Treatments					
	HT-Cotyledon	Cold-Cotyledon	PEG-Cotyledon	ABA-Cotyledon	NaCl-Cotyledon	Total-Cotyledon
NormFinder (Stab.)	<i>EF1a</i>	<i>UBQ</i>	<i>GAPDH</i>	<i>EF1a</i>	<i>GAPDH</i>	<i>GAPDH</i>
BestKeeper (CV)	<i>GAPDH</i>	<i>GAPDH</i>	<i>18S</i>	<i>18S</i>	<i>18S</i>	<i>18S</i>
BestKeeper (r^2)	<i>Actin</i>	<i>EF1a</i>	<i>GAPDH</i>	<i>EF1a</i>	<i>18S</i>	<i>GAPDH</i>
geNorm (M value)	<i>18S</i>	<i>Actin</i>	<i>18S</i>	<i>18S</i>	<i>18S</i>	<i>UBQ</i>
RankAggreg	<i>GAPDH</i>	<i>GAPDH</i>	<i>GAPDH</i>	<i>EF1a</i>	<i>18S</i>	<i>GAPDH</i>

Abbreviation: HT: High Temperature treatment; Cold: Cold treatment; ABA: Absciscic Acid treatment; NaCl: Sodium Chloride treatment; PEG: Polyethylene Glycol treatment; Stab. : Stability; CV: Coefficient of Variance; r^2 : Coefficient of Determination.