

Supplementary Materials

Stepwise Optimization of the RT-qPCR Protocol and the Evaluation of Housekeeping Genes in Pears (*Pyrus bretschneideri*) under Various Hormone Treatments and Stresses



Figure S1. Amplified fragments of candidate reference genes shown by agarose gel electrophoresis on optimal PCR amplicons in pear leaves under control condition. Lanes 1, 2, 3, 4, 5, 6, 7 and 8 were the genes of *ACT*, *SKD1*, *YLS8*, *WDP*, *TUB*, *UBQ*, *GAPDH* and *EF1α*. M marker 2000 (in ascending order: 100, 250, 500, 750, 1000, 1500 and 2000 bp). *ACT*, actin; *SKD1*, suppressor of K⁺ transport growth defect 1; *YLS8*, thioredoxin-like protein 8; *WDP*, WD-repeat protein; *TUB*, tubulin; *UBQ*, ubiquitin; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *EF1α*, elongation factor 1 alpha.

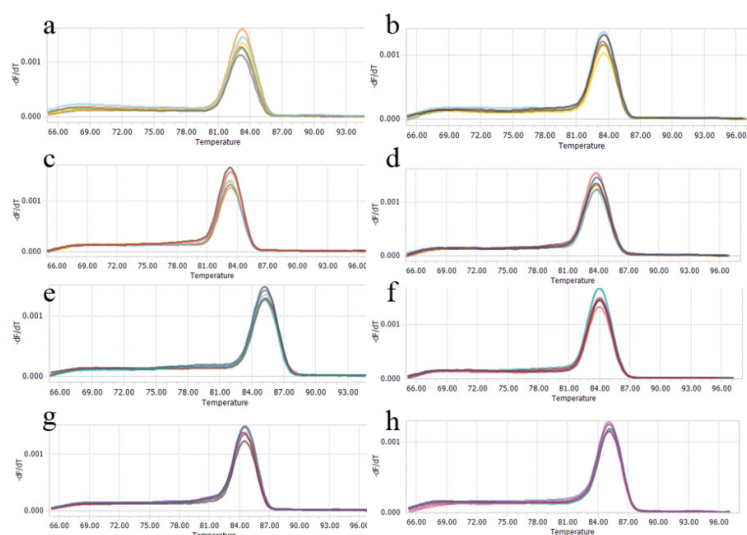


Figure S2. The analysis of specific primers and PCR efficiency for eight reference genes in pear leaves under control condition. Melting curves of reference genes showing single peaks. (a)*ACT*, (b)*SKD1*, (c)*YLS8*, (d)*WDP*, (e) *TUB*, (f)*UBQ*, (g) *GAPDH*, (h)*EF1α*.

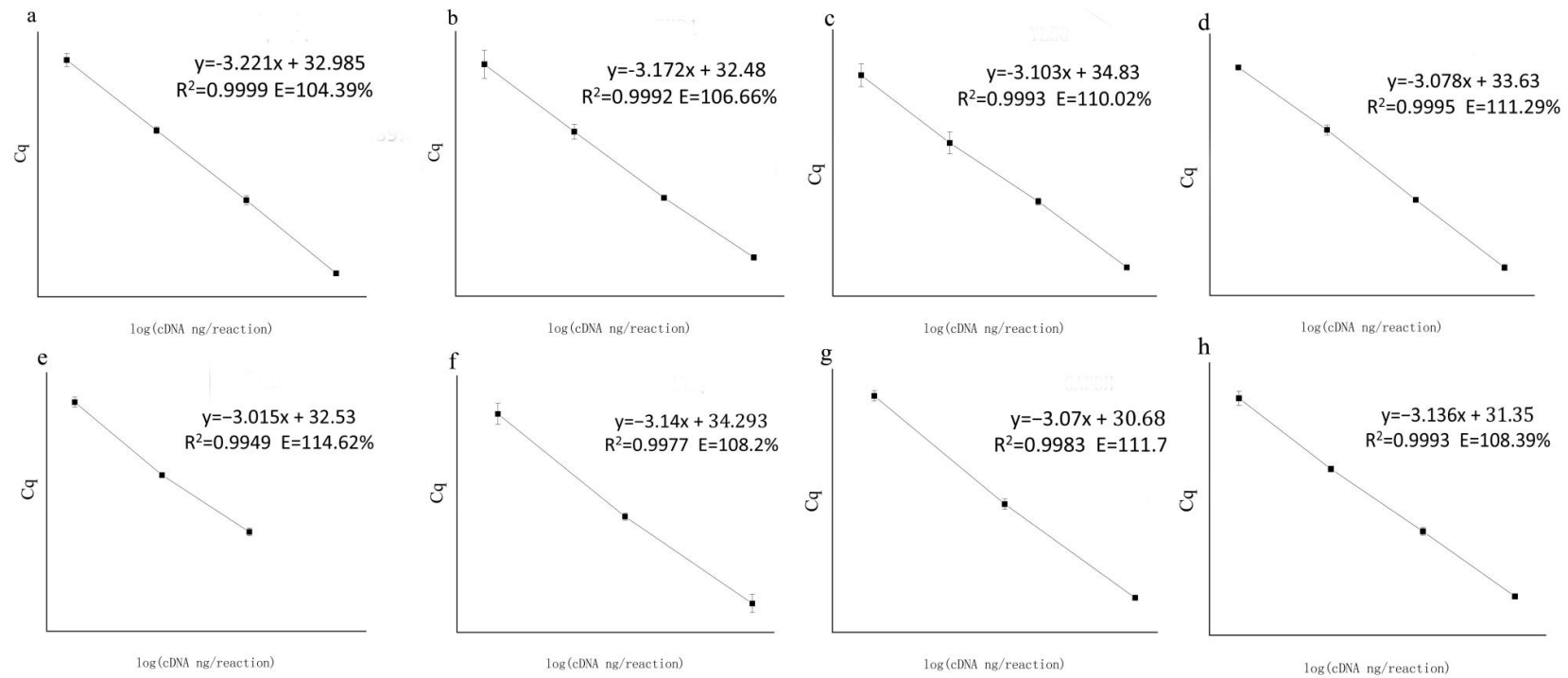


Figure S3. The plot of the averaged Cq values of three technical replicates against the Log (cDNA in ng/reaction) for optimizing qPCR conditions for each candidate reference genes in pears. One of the data cDNA concentration might have been omitted in order to obtain $R^2 \geq 0.99$ and $E = 100 \pm 10\%$. **a** ACT, **b** SKD1, **c** YLS8, **d** WDP, **e** TUB, **f** UBQ, **g** GAPDH, **h** EF1α.

Table S1. Primer sequences used for qRT-PCR and the details of optimized protocol.

Gene	Sequence ID	Primer sequences (5' –3')	Primer length	Tm	GC%	Amplicon length(bp)	annealing Temperature (° C)	Ct values of qPCR under different primers concentration(250/300/350/400mM)
<i>ACT</i>	GU830958	GTGCCCAGAAGTCCTCTTCC	20	60.04	60.00	139	60	26.94/26.75/26.79/ 26.74
		CCCACCACTAAGCACGATGT	20	60.04	55.00			
<i>SKD1</i>	XM009366023	CTTCCGCCTCCTATCAC	17	53.46	58.82	127	60	25.76/25.74/25.86/ 25.73
		TTCATCACCTTCCTCT	17	50.86	47.06			
<i>YLS8</i>	XM009353468	TGAGGTGCTGGCTTCTGT	18	58.43	55.56	119	60	28.42/ 28.17 /28.29/28.51
		TGACCGTTGATGGATCGTA	19	55.86	47.37			
<i>WDP</i>	Pbr038544	TCCACCGGCGAAGATATCAG	20	59.33	55.00	85	60	26.66/26.75/26.46/ 26.39
		CAGAAGGTTTCGGCTCCATCA	20	59.75	55.00			
<i>TUB</i>	Pbr042345	GTTCCAGAGCTCACTCAGCA	20	59.68	55.00	114	60	26.06/26.14/ 25.98 /26.02
		GGTGCTCATCTTACCACGGA	20	59.46	55.00			
<i>UBQ</i>	Pbr008614	GCCGAATCTCCCCCAATCTC	20	60.25	60.00	88	60	27.89/27.65/ 27.4 /27.6
		AGGGTTATCGTCTTCCCCGT	20	60.33	55.00			
<i>GAPDH</i>	Pbr036263	AGGGTTATCGTCTTCCCCGT	20	60.33	55.00	118	60	24.88/ 24.45 /24.77/24.78
		CCCTCAACAATCCCAAACC	19	56.07	52.63			
<i>EF1α</i>	XM_009376302	GCATCCAACCTCAAGGACGA	20	60.04	55.00	91	60	25.14 /25.36/25.48/25.29
		CGTTACCGATCTGACCAGGG	20	59.90	60.00			

Table S2. The Ct value of the reference genes of pear leaves under various experimental treatments. Ct, the average Ct value of each candidate reference gene under different conditions. SD, the standard deviation. CV, coefficient of variation. Three biological replicates per sample were used. **A** under hormones treatments. **B** under biotic and abiotic stresses treatments.

A															
Gene	MOCK			SA			MeJA			ETH			ABA		
	Ct	SD	CV (%)	Ct	SD	CV (%)	Ct	SD	CV (%)	Ct	SD	CV (%)	Ct	SD	CV (%)
<i>ACT</i>	21.68	0.84	3.88	23.55	1.73	7.35	27.17	0.91	3.35	24.29	2.42	9.97	23.20	2.86	12.31
<i>SKDI</i>	20.73	0.60	2.89	22.03	1.29	5.88	23.55	1.98	8.40	20.94	0.50	2.37	20.12	0.98	4.89
<i>YLS8</i>	22.72	0.23	1.02	24.23	1.64	6.76	26.29	1.95	7.43	23.54	1.61	6.84	22.81	1.88	8.24
<i>WDP</i>	22.36	0.64	2.85	23.91	1.71	7.15	25.95	1.40	5.38	23.49	1.23	5.22	22.47	1.54	6.87
<i>TUB</i>	21.53	0.76	3.55	22.97	1.84	8.00	26.34	1.12	4.24	24.20	1.43	5.92	23.29	1.63	6.98
<i>UBQ</i>	23.59	1.53	6.49	25.61	2.51	9.81	28.40	1.35	4.75	25.85	2.05	7.94	24.69	2.58	10.45
<i>GAPDH</i>	21.56	1.68	7.78	23.76	2.51	10.55	27.70	0.50	1.80	25.41	2.87	11.31	24.09	3.35	13.89
<i>EF1α</i>	20.53	1.37	6.65	22.49	2.25	10.00	26.34	0.86	3.27	22.91	3.45	15.08	21.97	3.93	17.88
B															
Gene	<i>V. nashicola</i>			Cold			Heat			Water-deficit			Salinity		
	Ct	SD	CV (%)	Ct	SD	CV (%)	Ct	SD	CV (%)	Ct	SD	CV (%)	Ct	SD	CV (%)
<i>ACT</i>	22.68	1.64	7.22	27.73	4.09	14.74	28.01	2.59	9.26	27.60	2.66	9.65	27.02	3.44	12.75
<i>SKDI</i>	21.39	1.07	5.00	23.42	2.75	11.73	22.42	2.92	13.04	26.46	1.75	6.61	23.76	2.67	11.24
<i>YLS8</i>	23.56	1.28	5.43	28.38	3.59	12.65	27.20	4.24	15.57	26.83	2.01	7.47	29.35	4.35	14.83
<i>WDP</i>	22.83	1.50	6.58	27.31	3.27	11.97	26.48	4.36	16.48	26.00	1.90	7.32	28.30	3.88	13.71
<i>TUB</i>	22.26	1.70	7.64	26.86	3.16	11.77	27.35	5.19	18.99	25.16	1.50	5.97	27.76	3.86	13.91
<i>UBQ</i>	24.63	2.24	9.09	28.30	2.13	7.52	28.39	3.55	12.50	27.02	0.94	3.46	29.33	5.22	17.80
<i>GAPDH</i>	23.30	2.62	11.22	28.25	2.65	9.38	28.10	3.09	10.98	27.17	1.01	3.72	28.69	2.71	9.46
<i>EF1α</i>	21.78	2.18	10.02	27.05	2.88	10.65	27.98	4.93	17.62	25.55	1.74	6.80	28.41	4.24	14.93