

Comparison of TaqMan and SYBR Green qPCR Methods for Quantitative Gene Expression in Tung Tree Tissues

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ABSTRACT: Quantitative real-time-PCR (qPCR) is widely used for gene expression analysis due to its large dynamic range, tremendous sensitivity, high sequence specificity, little to no postamplification processing, and sample throughput. TaqMan and SYBR Green qPCR are two frequently used methods. However, direct comparison of both methods using the same primers and biological samples is still limited. We compared both assays using seven RNAs from the seeds, leaves, and flowers of tung tree (*Vernicia fordii*), which produces high-value industrial oil. High-quality RNA were isolated from tung tissues, as indicated by a high rRNA ratio and RNA integrity number. qPCR primers and TaqMan probes were optimized. Under optimized conditions, both qPCR gave high correlation coefficient and similar amplification efficiency, but TaqMan qPCR generated higher y -intercepts than SYBR Green qPCR, which overestimated the expression levels regardless of the genes and tissues tested. This is validated using well-known *Dgat2* and *Fadx* gene expression in tung tissues. The results demonstrate that both assays are reliable for determining gene expression in tung tissues and that the TaqMan assay is more sensitive but generates lower calculated expression levels than the SYBR Green assay. This study suggests that any discussion of gene expression levels needs to be linked to which qPCR method is used in the analysis.

KEYWORDS: oil biosynthetic gene expression, quantitative real-time PCR, SYBR Green, TaqMan, tung tree

■ INTRODUCTION

Quantitative real-time-PCR (qPCR) is widely used for gene expression analysis due to its large dynamic range, tremendous sensitivity, high sequence specificity, little to no postamplification processing, and sample throughput. TaqMan and SYBR Green qPCR assays are two frequently used qPCR techniques. TaqMan uses a fluorogenic single-stranded oligonucleotide probe that binds only the DNA sequence between the two PCR primers.¹ Therefore, only specific PCR product can generate a fluorescent signal in TaqMan PCR. The TaqMan probe principle relies on the 5'-3' exonuclease activity of Taq polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence and fluorophore-based detection. As in other real-time PCR methods, the resulting fluorescence signal permits quantitative measurements of the accumulation of the product during the exponential stages of the PCR; however, the TaqMan probe significantly increases the specificity of the detection. SYBR Green qPCR is widely used because of the ease in designing the assays and its relatively low setup and running costs. Unlike TaqMan fluorescent probes, SYBR Green dye intercalates into double-stranded DNA to monitor the amplification of the target gene specifically initiated by gene-specific primers.² One drawback of SYBR Green assay is that the dye is nonspecific, which can generate false positive signals if nonspecific products or primer-dimers are present in the assay. The other drawback of the SYBR Green assay is that the length of the amplicon also affects the intensity of the amplification. Direct comparison of both methods using the same biological samples and the same PCR primers is still limited.

Tung tree (*Vernicia fordii*) is a tropical plant with a very limited growing area in the southeastern United States.³ Tung

tree produces large seeds containing about 50–60% oil (dry weight basis) with approximately 80 mol % α -eleostearic acid (9*cis*,11*trans*,13*trans*-octadecatrienoic acid).⁴ Tung oil is readily oxidized due to the three unique conjugated double bonds in eleostearic acid. Dried tung oil is impervious to heat, moisture, dust, and many chemical challenges and, unlike other drying oils, does not darken with age. These properties underlie the value of tung oil as a drying ingredient in paints, varnishes, and other coatings and finishes. Most of the tung orchards in the southern United States were destroyed by hurricanes including Hurricanes Betsy in 1965, Camille in 1969, and Katrina and Rita in 2005. These losses spurred interest in trying to preserve a reliable domestic source of tung oil by transferring the genetic components of the tung oil biosynthetic pathway into traditional, temperate oilseeds.

Our project focuses on engineering oilseed crops to produce tunglike drying oils and other high-value industrial vegetable oils. A number of candidate genes have been identified for tung oil biosynthesis, including FADX, the diverged FAD2-like enzyme that catalyzes the formation of α -eleostearic acid from linoleic acid;⁵ diacylglycerol acyltransferases (DGAT), a group of hydrophobic membrane proteins responsible for the last and rate-limiting step of triacylglycerol (TAG) biosynthesis in eukaryotic organisms;^{6–9} cytochrome b5 (Cb5);¹⁰ cytochrome b5 reductase;¹¹ glycerol-3-phosphate acyltransferase (Gpat);¹² and oleosins (Ole).¹³ The Northern blotting technique demonstrates that *Dgat2* and *Fadx* genes are predominately

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Table 1. mRNA Analyzed by qPCR and the Nucleotide Sequences of qPCR Primers and TaqMan Probes

no.	mRNA	name	accession no.	amplicon	forward primer (5' to 3')	TaqMan probe (5' to 3')	reverse primer (5' to 3')
1	Cb5a	cytochrome b5	AY578727	73	ATTTCATCCACTGTTCCAGCAA	CGGACACACATCCCT- CCGAAGCA	TCTGAGCTCTTGTC- TGATTGTAGA
2	Dgat2	diacylglycerol acyltransferase	DQ356682	80	TGCATGTCGTGGTGGGTAGA	AAGCAAAATCCACAG- CCTACCCTGAA	TCTCTGTACTTCCGC- AACCT
3	Fadx	delta-12 fatty acid conjugase	AF525535	70	AAAAGGAAAGCAGAAGCGTGAA	CGAGTCCCACACACA- AAGCCTCCA	TGCTTTAATTGGCCA- AGAGTGA
4	Gpat8	glycerol-3- phosphate acyltransferase	FJ479753	69	CCTTTGCTGGGCTCAAGATC	CGACATCGAGCTGGT- TTCCCGC	CAGCATAAAACCTAG- GCAAAACAG
5	Lacs9	long chain acyl- CoA synthetase	unpublished	57	GGAAGGTTTCGAGCAGTTTTGG	AGGTCGCATCCGTTTT	GAGCACCTCCGAAA- GCA
6	Lpat2	lysophosphatidyl acyltransferase	unpublished	66	GCCGACCTATGAAGTCTCTTG- TG	CGTTACTCTTGGGC- GTGCCTGC	TCAGGGCCCCAAAACCA
7	Rpl19b	ribosomal protein 19b	FJ362591	70	GCGGAGAATGCGTGTCTG	CCTGCTGCGCAAATA- CCGGGAA	CATGTGCTTGTCAAT- TTTTTTGG

expressed in the developing tung seeds.^{5,7} SYBR Green qPCR showed that Fadx expression is restricted to tung leaves but increased in more mature seeds.¹⁴ This discrepancy deserves more study. In addition, less is known about the expression patterns of many other genes in tung seeds.

As a first step to profile gene expression in tung oil biosynthesis, we aimed to establish a reliable method for analyzing oil biosynthetic gene expression in tung tree tissues. We validated seven TaqMan and SYBR Green qPCR assays, optimized these assays, and compared both assays using cDNA from tung seeds, leaves, and flowers. Our data show that both assays are reliable for determining the expression of these genes in tung tissues. The surprising finding is that the TaqMan assay is more sensitive but generates lower calculated expression levels than the SYBR Green assay regardless of the tissues and gene targets tested. Therefore, interpretation of qPCR results between these two assays requires caution.

MATERIALS AND METHODS

Tung mRNA Sequences. Tung mRNA sequences were obtained from the National Center for Biotechnology Information (NCBI)'s nonredundant protein sequence databases (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).⁶ Additional tung mRNA sequences were obtained from in-house sequencing projects. The names of mRNAs analyzed by qPCR and their corresponding GenBank accession numbers are presented in Table 1.

qPCR Primers and TaqMan Probes. The qPCR primers and TaqMan probes were designed using Primer Express software (Applied Biosystems, Foster City, CA). They were synthesized by Biosearch Technologies, Inc. (Navato, CA). The amplicon sizes and the nucleotide sequences (5' to 3') of the forward primers, TaqMan probes [Dual-Labeled tetrachlorofluorescein (TET)-Black Hole Quencher-1 (BHQ1)], and reverse primers, respectively, are described in Table 1.

RNA Isolation. Tung trees were grown in the American Tung Oil Corporation orchard in Lumberton, MS. Tung seeds were excised from the trees, and kernels were immediately frozen in liquid N₂ and stored at -80 °C. Tung seeds were ground into powder with a mortar and pestle under liquid nitrogen. The total RNA were isolated by a Spectrum Plant Total RNA Kit (Sigma, St. Louis, MO) according to the manufacturer's instructions. The total RNA from tung seeds, leaves, and flowers was also extracted using the hot borate method.¹⁵ RNA concentrations and integrity were determined using a RNA 6000 Nano Assay Kit and the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) with RNA 6000 Ladder as the standards.¹⁶ Typical electropherograms and chromatograms of the total RNA are shown in Figure 1.

cDNA Synthesis. The cDNA were synthesized from total RNA using SuperScript II reverse transcriptase.¹⁷ The cDNA synthesis mixture (20 μL) contained 5 μg of total RNA, 2.4 μg of oligo(dT)₁₂₋₁₈ primer, 0.1 μg of random primers, 500 μM dNTPs, 10 mM DTT, 40 u RNaseOUT, and 200 u SuperScript II reverse transcriptase in 1X first-strand synthesis buffer (Life Technologies, Carlsbad, CA). The cDNA synthesis reaction proceeded at 42 °C for 50 min. The cDNA was diluted with water to 1–5 ng/μL before qPCR analyses.

qPCR Analysis. The TaqMan qPCR reaction mixture contained variable amounts of total RNA-derived cDNA, the forward primer, reverse primer, TaqMan probe, and 1X Absolute QPCR Mix (ABgene House, Epsom, Surrey, United Kingdom).¹⁶ The SYBR Green qPCR reaction mixture contained variable amounts of total RNA-derived cDNA, forward primer, reverse primer, and 1X iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). The reactions in 96-well plates were performed with CFX96 real-time system-C1000 Thermal Cycler (Bio-Rad Laboratories). The thermal cycle conditions for TaqMan assay were as follows: 2 min at 50 °C and 15 min at 95 °C (this step is required for the activation of Thermo-Start DNA polymerase, a chemically modified hot-start version of Thermoprime Taq DNA polymerase, which prevents nonspecific amplification), followed by 50 cycles at 95 °C for 15 s and 60 °C for 60 s. The thermal cycle conditions for SYBR Green assay were as follows: 3 min at 95 °C, followed by 40 cycles at 95 °C for 10 s, 65 °C for 30 s, and 72 °C for 30 s. The 2^{-ΔCT} method of relative quantification was used to determine the fold change in expression.¹⁸ The expression of tung 60s ribosomal protein L19 (Rpl19b, GenBank accession no. FJ362591) was used as the reference mRNA in the qPCR analyses because its expression is highly stable among the tung seeds, leaves, and flowers (refer to the Results and Discussion for details.)

TaqMan and SYBR Green qPCR Optimization. The qPCR reactions contained 5 ng of RNA-equivalent cDNA from tung seed, leaf, and flower. For TaqMan qPCR primer optimization, the mixtures contained variable primer concentrations (10, 50, 100, 200, and 400 nM) and a fixed probe concentration (200 nM). For TaqMan qPCR probe optimization, the mixtures contained variable probe concentrations (10, 50, 100, 200, and 400 nM) and a fixed primer concentration (200 nM each of the forward and reverse primers). For SYBR Green qPCR primer optimization, the reaction mixtures contained variable primer concentrations (10, 50, 100, 200, and 400 nM).

TaqMan and SYBR Green qPCR Efficiency. The qPCR reactions contained variable template concentrations (0.05, 0.5, 2.5, 5, 12.5, and 25 ng) of RNA-equivalent cDNA from tung seeds, leaves, and flowers. The assay mixtures contained optimized concentrations (200 nM) for the primers with or without TaqMan probe. The amplification efficiency of qPCR assay is estimated on the basis of the equation $E = (10^{-1/\text{slope}} - 1) \times 100$.¹⁹

Statistical Analysis. The means and standard deviations were determined from two to four assays of each mRNA analyzed by both qPCR methods.

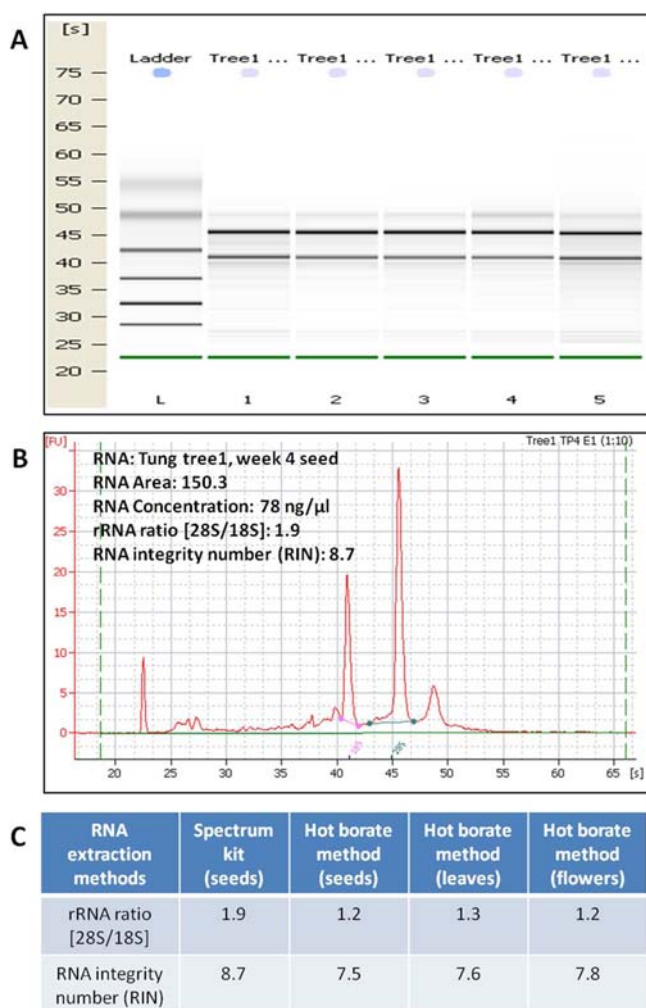


Figure 1. Integrity of RNA isolated from tung seeds, leaves, and flowers. The total RNA was isolated from tung tree tissues by a Spectrum Plant Total RNA Kit and hot borate method. RNA concentrations and integrity were determined using a RNA 6000 Nano Assay Kit and the Bioanalyzer 2100 with RNA 6000 Ladder as the standards. A typical electropherogram and a chromatogram of the total RNA are shown. (A) Electropherogram of RNA ladder (lane L) and total RNA isolated by a Spectrum Plant Total RNA Kit from tung seeds collected weeks 1 (lane 1), 2 (lane 2), 3 (lane 3), 4 (lane 4), and 5 (lane 5). (B) Chromatogram of total RNA isolated from week 4 tung tree seed by a Spectrum Plant Total RNA Kit. The two most abundant peaks are 18S and 28S rRNA. (C) Comparison of the quality of RNA isolated by a Spectrum Plant Total RNA Kit and the hot borate method.

RESULTS AND DISCUSSION

RNA Isolation. The total RNA were isolated from developing tung seeds that were rapidly accumulating eleostearic acid. They were also isolated from tung leaves and flowers and used as comparison to the seeds. The RNA isolated by the Spectrum Plant Total RNA Kit were shown to be high quality as determined by the sharp 18S and 28S rRNA bands on the electropherogram (Figure 1A) and sharp peaks on the chromatogram (Figure 1B). The spectrum method generated a higher rRNA ratio (28S/18S = 1.9) and RNA integrity number (RIN = 8.7) than those isolated by the hot borate method with a rRNA ratio (28S/18S = 1.2) and the RNA integrity number (RIN = 7.5) (Figure 1C). RIN is generated by an algorithm for assigning integrity values to RNA measurements, which is applied to electrophoretic RNA measurements and based on a

combination of different features that contribute information about the RNA integrity to provide a more robust universal measure.²⁰ These results demonstrate that the Spectrum Plant Total RNA Kit method is suitable for RNA extraction from oil-rich tung seeds and is a better method than the hot borate method¹⁵ previously used for RNA extraction from tung seeds.⁷

TaqMan qPCR Optimization. The concentrations of TaqMan primers were optimized using 5 ng of RNA-equivalent cDNA from tung seeds, leaves, and flowers (Figure 2A–C). qPCR reactions contained 10, 50, 100, 200, and 400 nM each of forward primer and reverse primer and a fixed probe concentration at 200 nM. The C_T value of qPCR amplification was much higher when the primer pair concentrations were only 10 nM, but the C_T values were similar when higher concentrations of primer pairs were used in the assays (the higher the C_T value, the less the expression level). One hundred nanomolar primer pair concentrations and above saturated the TaqMan qPCR reactions. The concentrations of TaqMan probes were also optimized using the same amount of cDNA from tung seeds, leaves, and flowers (Figure 2D–F). These qPCR reactions contained 10, 50, 100, 200, and 400 nM TaqMan probe and fixed primer pair concentrations at 200 nM. The C_T value of qPCR amplification was higher when the probe concentrations were only 10 nM, but the C_T values were similar after 100 nM probes or higher concentrations were used in the qPCR reactions. Similar qPCR results were obtained using cDNA from tung seeds (Figure 2A,D), leaves (Figure 2B,E), and flowers (Figure 2C,F). These results indicate that 100 nM or above of the forward primer, reverse primer, and TaqMan probe are optimal concentrations for the TaqMan qPCR assay.

SYBR Green qPCR Optimization. The concentrations of SYBR Green qPCR primers were optimized using 5 ng of RNA-equivalent cDNA from tung seeds, leaves, and flowers. qPCR reactions contained 10, 50, 100, 200, and 400 nM each of forward primer and reverse primer. Figure 3 shows that qPCR amplification as measured by C_T value was undetectable when 10 nM primers were used, and the C_T value was much higher when the primer pair concentrations were only 50 nM, but C_T values were similar when higher concentrations of primer pairs were used. Two hundred nanomolar primer pair concentrations saturated the qPCR reactions. Similar qPCR results were obtained using cDNA from tung seeds (Figure 3A), leaves (Figure 3B), and flowers (Figure 3C). Melt curve analyses showed a single melt peak in each qPCR melt curve for each target qPCR amplification using RNA from tung seeds (Figure 3D), leaves (Figure 3E), and flowers (Figure 3F). These results indicate that 200 nM or above of the forward primer and reverse primer are optimal concentrations for SYBR Green qPCR assay, which amplifies mRNA-specific PCR products. However, in this study, the TaqMan assay was more sensitive than the SYBR Green assay since qPCR amplification products were detected with TaqMan assays when using the lowest concentrations of the primer pairs (10 nM) and saturated at 100 nM (Figure 2 vs Figure 3).

TaqMan and SYBR Green qPCR Efficiency. The optimized primer and probe concentrations (200 nM) were used for estimating qPCR efficiency under variable template concentrations (0.05, 0.5, 2.5, 5, 12.5, and 25 ng) of RNA-equivalent cDNA from tung seeds. Figure 4A shows the comparison of C_T values versus amounts of cDNA using the primer pair and probe for Rpl19b with TaqMan and SYBR Green qPCR assays. Both assays gave a high correlation coefficient ($r^2 > 0.99$). The TaqMan qPCR assay generated a

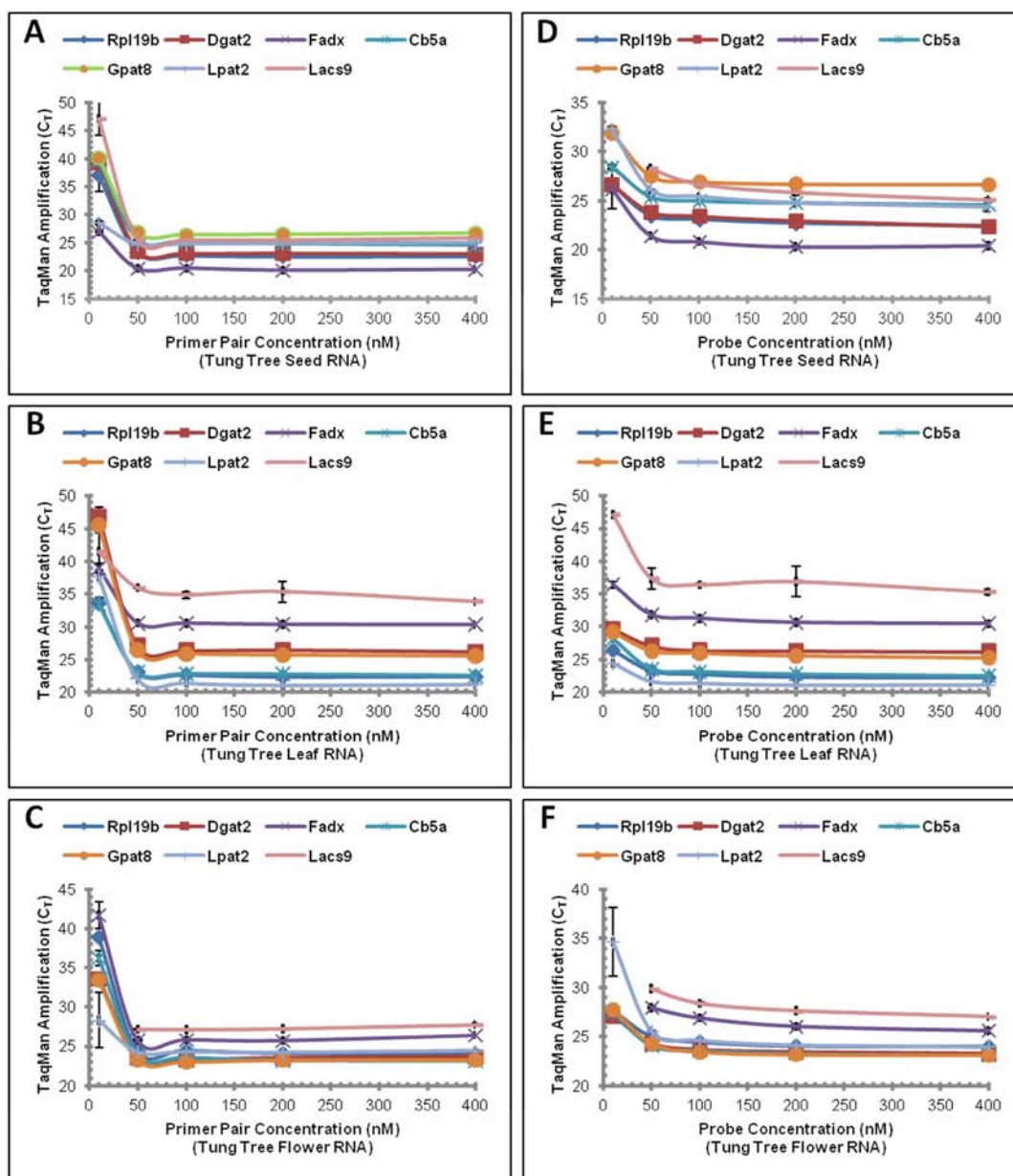


Figure 2. TaqMan qPCR optimization. TaqMan qPCR reactions contained 5 ng of RNA-equivalent cDNA from tung seeds (A and D), leaves (B and E), and flowers (C and F), forward primer, reverse primer, TaqMan probe, and Absolute QPCR Mix. The reactions were performed with CFX96 real-time system-C1000 Thermal Cycler. Left panels (primer optimization), the mixtures contained variable primer pair concentrations and fixed probe concentration (200 nM). Right panels (probe optimization), the mixtures contained variable probe concentrations and fixed primer pair concentration (200 nM).

greater slope than that of the SYBR Green qPCR assay, suggesting a higher sensitivity with the TaqMan qPCR assay. Similar trends were generated using Dgat2 primer pair and probe (Figure 4B) and the qPCR primer pairs and TaqMan probes for the other mRNAs (Table 2). These two qPCR assays gave a similar amplification efficiency (93 ± 3 and $89 \pm 3\%$, $n = 7$, respectively), but the TaqMan assay generated higher y -intercepts than those of SYBR Green qPCR assays (Table 2). The y -intercept corresponds to the theoretical limit of detection of the reaction, or the C_T value expected if the lowest copy number of target molecules denoted on the x -axis gave rise to statistically significant amplification. These results suggest that both qPCR assays are reliable methods for analyzing oil biosynthetic gene expression in tung tissues and

confirm the conclusion from the previous section that TaqMan qPCR is more sensitive than SYBR Green qPCR.

Reference Gene Expression. The expression of genes coding for ribosome proteins is highly stable, and they are widely used as reference genes for calculating gene expression.^{21–24} The expression of tung 60s Rpl19b was analyzed using RNA isolated from tung seeds, leaves, and flowers under an optimized primer pair and probe concentration at 200 nM. The C_T values of TaqMan assay for tung seeds, leaves, and flowers were 22.35 ± 0.01 , 22.90 ± 0.05 , and 22.46 ± 0.10 , respectively (Table 3). The C_T values of TaqMan assay among the three tissues were 22.57 ± 0.29 (Table 3). The C_T values of SYBR Green assay for tung seeds, leaves, and flowers were 20.70 ± 0.52 , 20.14 ± 0.18 , and 20.64 ± 0.39 , respectively (Table 3). The C_T values of SYBR Green assay

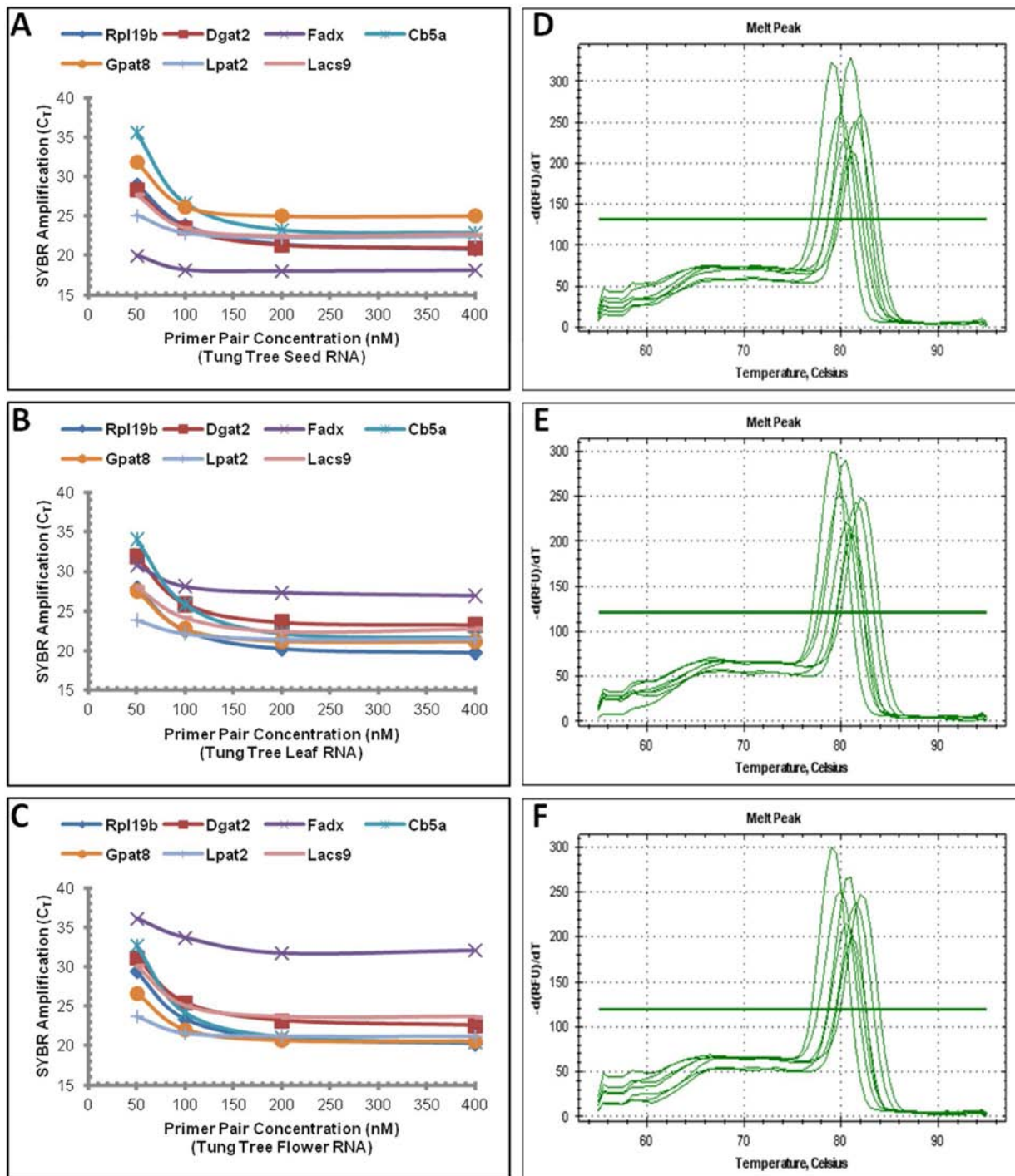


Figure 3. SYBR Green qPCR optimization. The SYBR Green qPCR reactions contained 5 ng of RNA-equivalent cDNA from tung seeds (A and D), leaves (B and E), and flowers (C and F), forward primer, reverse primer, and iQ SYBR Green Supermix. The reactions were performed with CFX96 real-time system-C1000 Thermal Cycler. The reaction mixtures contained variable primer pair concentrations. Left panels, primer optimization; right panels, melt curve analysis.

among the three tissues were 20.49 ± 0.31 (Table 3). These results demonstrate that Rpl19b is very stably expressed in the three tissues and is suitable as a reference mRNA in the qPCR analyses.

SYBR Green qPCR Overestimated Gene Expression Levels than TaqMan qPCR. The C_T values from the TaqMan qPCR assay were generally lower than those from the SYBR qPCR assay regardless of the amount and sources of cDNA

from tung tissues used in the analyses (Figure 2 vs Figure 3). To quantify the differences between these two qPCR assays, the relative expression levels of selected genes were compared using data generated by TaqMan and SYBR Green qPCR assays under the optimized primers and probe concentrations (200 nM) and 5 ng of RNA-equivalent cDNA from tung tissues. To our surprise, the expression levels of all of the selected genes generated by SYBR Green qPCR

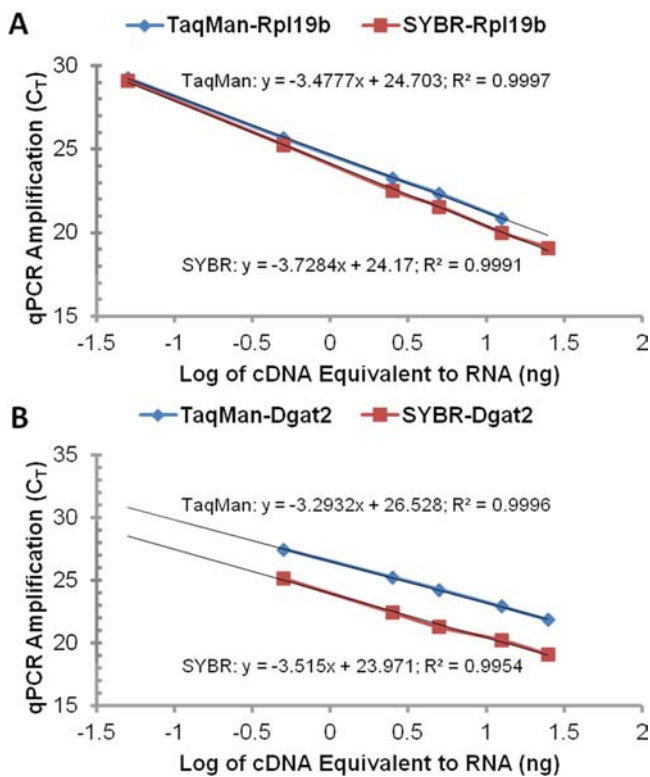


Figure 4. Comparison of TaqMan and SYBR Green qPCR efficiency. TaqMan qPCR reaction mixtures contained variable concentrations of RNA-equivalent cDNA from tung seeds, the optimized concentrations of each primer and probe (200 nM), and Absolute QPCR Mix. The SYBR Green qPCR reaction mixture contained variable concentrations of RNA-equivalent cDNA from tung seeds, the optimized concentrations of each primer (200 nM), and iQ SYBR Green Supermix. The results of Rpl19b (A) and Dgat2 (B) are shown in the figure. The results of the other qPCR assays are presented in Table 2.

assay were at least 2-fold higher than those obtained by TaqMan qPCR assay regardless of the RNA isolated from tung seeds (Figure 5A), leaves (Figure 5B), or flowers (Figure 5C).

The above differences on the calculated gene expression levels between TaqMan and SYBR Green qPCR assays could be conveniently explained by the different chemistry employed by the two assays. TaqMan uses a fluorogenic single-stranded oligonucleotide probe that binds only the DNA sequence between the two PCR primers.¹ Therefore, only specific PCR product can generate a fluorescent signal in TaqMan qPCR. However, the SYBR Green dye intercalates into double-

Table 3. Reference Gene Expression in Tung Tree Tissues

	C_T			SD
	Rpl19b	Rpl19b	Rpl19b mean	
	TaqMan qPCR			
seed	22.35	22.34	22.35	0.01
leaf	22.87	22.94	22.90	0.05
flower	22.39	22.53	22.46	0.10
tissue mean	22.54	22.61	22.57	
SD			0.29	
	SYBR Green qPCR			
seed	21.07	20.34	20.70	0.52
leaf	20.27	20.01	20.14	0.18
flower	20.91	20.36	20.64	0.39
tissue mean	20.75	20.24	20.49	
SD			0.31	

stranded DNA to monitor the amplification of target gene specifically initiated by gene-specific primers.² Therefore, SYBR Green qPCR assays can generate false positive signals if nonspecific products or primer-dimers are present in the assay. Melting curve analysis of SYBR Green qPCR assays is widely used to determine if nonspecific products are formed during qPCR amplification.¹⁴ Our analyses showed that all assays generated single melting peaks for the tested mRNA expression in tung seeds, leaves, and flowers (Figure 3D–F), suggesting that SYBR Green qPCR assays produced mRNA-specific amplification products in our SYBR Green qPCR assays. These results suggest that overestimation of expression levels of these genes in tung seeds, leaves, and flowers by the SYBR Green qPCR assay could not be simply explained by the employed dye-binding chemistries in the PCR amplification.

Validation of TaqMan and SYBR Green qPCR for Quantitative Gene Expression in Tung Tissues. We compared the relative expression levels of the selected genes among the three different tissues to validate our qPCR methods. The TaqMan qPCR assay showed that the Rpl19b expression level was similar among the three tissues (Figure 6A). Dgat2 and Fadx were predominantly expressed in tung seeds, whereas Gpat8 was mainly expressed in tung leaves and flower (Figure 6A). Cb5a and Lpat2 expression levels were higher in tung flowers and leaves, but Lacs9 was mainly expressed in tung seeds and leaves (Figure 6A). Almost identical expression patterns of the selected genes in the three tung tree tissues were observed using SYBR Green qPCR method (Figure 6B).

In our studies, both qPCR methods demonstrate that Dgat2 and Fadx are predominately expressed in the developing tung

Table 2. TaqMan and SYBR qPCR Efficiency

mRNA	TaqMan				SYBR Green			
	slope	y-intercept	correlation coefficient	efficiency $E = [10(1/-S) - 1] \times 100\%$	slope	y-intercept	correlation coefficient	efficiency $E = [10(1/-S) - 1] \times 100\%$
Rpl19b	-3.4777	24.702	0.9997	94	-3.7284	24.170	0.9991	85
Dgat2	-3.2932	26.528	0.9996	101	-3.5150	23.971	0.9954	92
Fadx	-3.5441	23.513	0.9991	91	-3.7866	21.735	0.9962	84
Lpat2	-3.5241	27.983	0.9991	92	-3.6946	24.811	0.9943	87
Gpat8	-3.4979	28.771	0.9988	93	-3.5855	26.326	0.9980	90
Cb5a	-3.6611	27.353	0.9990	87	-3.5577	26.595	0.9921	91
Lacs9	-3.4636	27.985	0.9920	95	-3.5601	23.919	0.9976	91
mean \pm SD ($n = 7$)				93 \pm 3%				89 \pm 3%

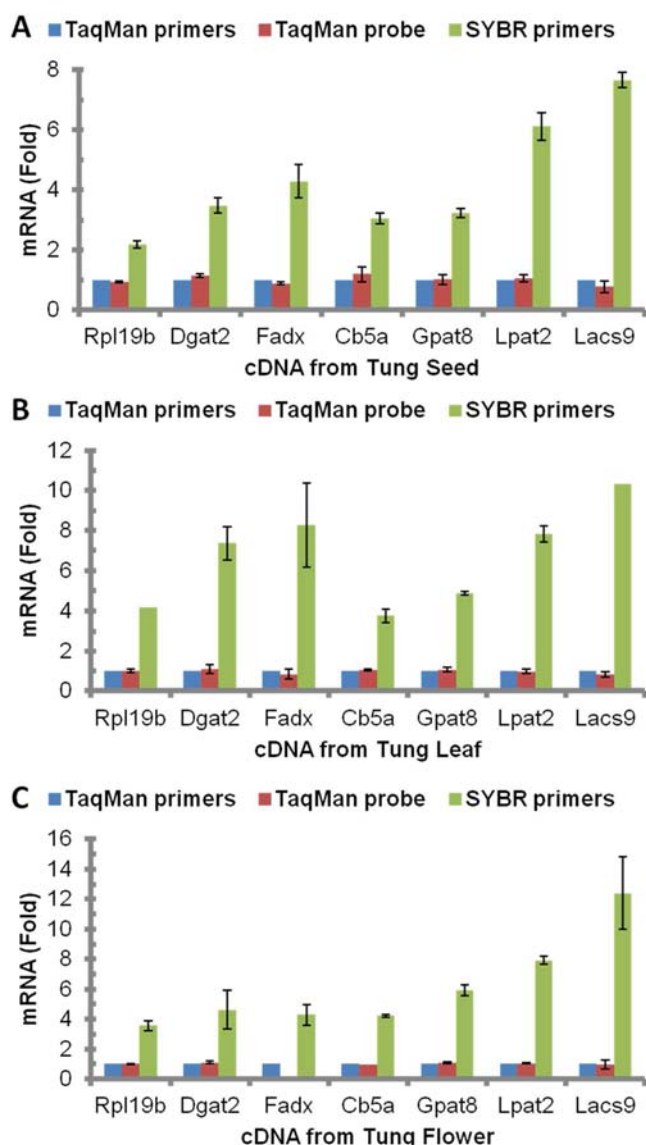


Figure 5. Comparison of TaqMan and SYBR Green qPCR for quantitative gene expression. qPCR reaction mixtures contained 5 ng of RNA-equivalent cDNA from tung seeds (A), leaves (B), and flowers (C), the optimized concentrations (200 nM) of each primer (SYBR Green assay) and probe (TaqMan assay), and Absolute QPCR Mix for TaqMan assay or iQ SYBR Green Supermix for SYBR Green assay. Inside each panel, TaqMan primers refer to the fact that both primers were added first when the TaqMan assay was set up; TaqMan probe refers to the fact that the probe was added first when the assay was set up. This is to test the reproducibility of TaqMan assays in both ways. Means and standard deviations of 2–4 assays are presented.

seeds. These results are in agreement with our previous analyses using the Northern blotting method.^{5,7} A recent study showed by SYBR Green qPCR that Fadx expression is restricted to tung leaves, but the level is increased in more mature tung seeds.¹⁴ We do not have a good explanation for the discrepancy between these studies. The expression of the other genes in tung tree is unknown at present. Nonetheless, the results from our validation study demonstrate that both TaqMan and SYBR Green qPCR methods are reliable for quantitative gene expression in tung tree tissues.

In summary, these results show that the Spectrum Plant Total RNA Kit is suitable for RNA extraction from oil-rich tung

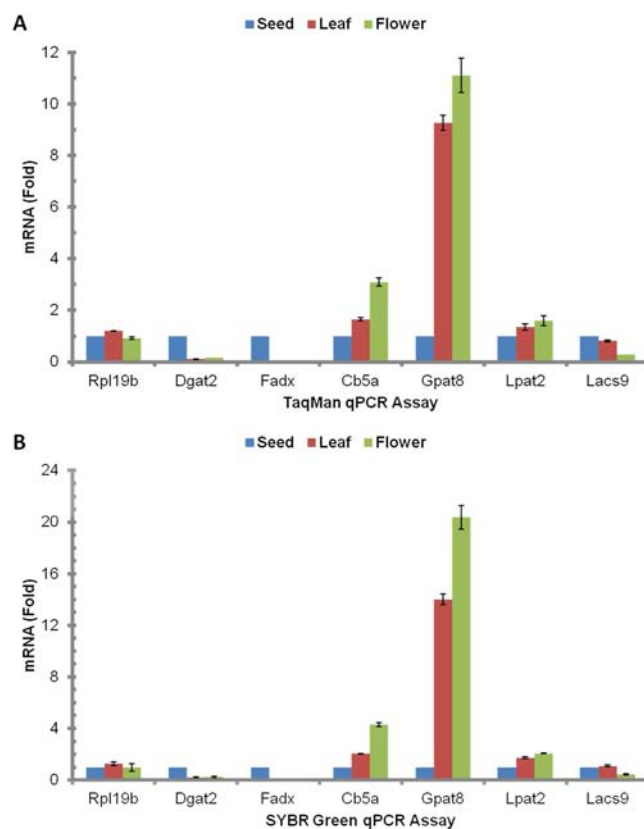


Figure 6. Validation of TaqMan and SYBR Green qPCR for quantitative gene expression. (A) TaqMan qPCR assays and (B) SYBR Green qPCR Assays. qPCR reaction mixtures contained 5 ng of RNA-equivalent cDNA from tung seeds, leaves, and flowers, the optimized concentrations (200 nM) of each primer (SYBR Green assay) and probe (TaqMan assay), and Absolute QPCR Mix for TaqMan assay or iQ SYBR Green Supermix for SYBR Green assay. Means and standard deviations of 2–4 assays are presented.

tissues and better than the hot borate method. Optimization studies indicate that 200 nM qPCR primers and TaqMan probes are sufficient for both TaqMan and SYBR Green qPCR assays using tung tissues. Under optimized assay conditions using variable template concentrations, both qPCR assays give high correlation coefficient and similar amplification efficiency, but SYBR Green qPCR overestimates the expression levels. The results presented in the paper demonstrate that both TaqMan and SYBR green qPCR methods are reliable for determining the oil biosynthetic gene expression in tung tissues, although the TaqMan qPCR assay is more sensitive but generates lower calculated expression levels than the SYBR Green qPCR assay. Our results suggest that any discussion of gene expression levels should mention which qPCR method is used for the expression analysis.

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Notes

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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ABBREVIATIONS USED

Cb5, cytochrome b5; Dgat, diacylglycerol acyltransferase; Fad, fatty acid desaturase; Gpat, glycerol-3-phosphate acyltransferase; Lacs, long-chain acyl-CoA synthetase; Lpat, lysophosphatidyl acyltransferase; Ole, oleosin; qPCR, quantitative real-time PCR; Rpl19b, ribosomal protein 19b; TAG, triacylglycerol

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