Hindawi
Journal of Sensors
Volume 2023, Article ID 9815293, 1 page
https://doi.org/10.1155/2023/9815293

Retraction

Retracted: Selection of Optimum Internal Control Genes for RT-qPCR Analysis of Schisandra chinensis under Four Hormone Treatments

Journal of Sensors

Received 19 December 2023; Accepted 19 December 2023; Published 20 December 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Manipulated or compromised peer review

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

References

[1] X. Liu, L. Zhang, and S. Yang, "Selection of Optimum Internal Control Genes for RT-qPCR Analysis of Schisandra chinensis under Four Hormone Treatments," *Journal of Sensors*, vol. 2022, Article ID 9299289, 10 pages, 2022.

Hindawi
Journal of Sensors
Volume 2022, Article ID 9299289, 10 pages
https://doi.org/10.1155/2022/9299289

Research Article

Selection of Optimum Internal Control Genes for RT-qPCR Analysis of *Schisandra chinensis* under Four Hormone Treatments

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Received 2 August 2022; Revised 23 August 2022; Accepted 2 September 2022; Published 25 September 2022

Academic Editor: Sweta Bhattacharya

qRT-PCR technology is now one of the commonly used methods to study gene expression levels. The selection of accurate reference gene detection is an essential work before gene expressing analysis. In the current study, 8 candidate internal control genes (ACTIN, TUBA, GAPDH, UBC, MUB, TIP41, APX, and CAPA) were selected, and four statistical algorithms were used to evaluate their stability under different hormone treatments. The results confirmed that when using one internal control genes, TUBA emerged as the first ranking internal control gene in all experimental groups. When using two internal control genes, TUBA and MUB, were the most acceptable internal control genes for the GA3 treatment group; TUBA and GAPDH were identified as internal control genes in the IAA treatment group; TUBA and ACTIN were the most reliable combination in the ZT and ABA experimental groups; TUBA and TIP41 were recommended most suitable control genes in control group. Furthermore, the reliability of the internal control genes was further verified by the expression of GAG, a gene related to the development of *Schisandra chinensis*. The conclusion of this work will be helpful for the subsequent research on gene expression analysis of *Schisandra chinensis*.

1. Introduction

Quantitative real-time PCR (qRT-PCR) has become the most common assay for transcript and gene expressing levels due to its specificity, accuracy, efficiency, and high sensitivity [1, 2]. qRT-PCR results require data normalization by reliable internal control genes [3, 4]. As internal control genes, ideal ones are stably expressed in all tissues, and their expression is independent of environmental factors, experimental conditions, or other factors [5]. However, previous reports indicate that there were no such internal control genes, which can be applied to all plants and various experimental designs [6]. For example, different internal control genes were used under different experimental conditions in *Cocos nucifera L.* (Coconut) [7], *Momordica charantia* [8], *Bixa orellana* L. [9], *Salix matsudana* [10], and *Taihangia rupestris* [11]. For reliable and precise qRT-PCR results, it is therefore important to evaluate and choice beneficiary internal control genes under different experimental conditions [12].

Schisandra chinensis (*S. chinensis*) is a monoecious liana of the family Magnoliaceae. It grows in the eastern most parts of Russia, the Kuril Islands, southern Sakhalin, Korea, Japan, and northeastern China [13]. The dried ripe fruit of *S. chinensis* is a Chinese traditional herbal medicine, used for tonifying qi, promoting the production of body fluid, nourishing kidneys, and its calming and astringent properties [14, 15]. Since male and female flowers of *S. chinensis* grow on the same plant, the quantity and quality of female flowers determine the yield of *S. chinensis*. Therefore, improving the differentiation rate of female flowers is of great significance to improve the yield of *S. chinensis*.

Many studies have shown that exogenous hormones have regulatory effects on plant flower development [16, 17]. However, the research on how phytohormones regulate flower development is not deep enough to fully explain its regulatory mechanism. In this study, the effects of exogenous hormones on the differentiation of female flowers of *S. chinensis* were investigated by spraying different concentrations of exogenous hormones. But it does not appear to be a universal internal control gene that would be suitable for *S. chinensis* to our knowledge. For this reason, it is important to choose correct internal control genes when interpreting RT-qPCR results. We selected eight candidate internal control genes (ACTIN, TUBA, MUB, UBC, GAPDH, GAPA, TIP41, and APX) from the transcriptome data, and their stability was evaluated. Furthermore, we used geNorm [18], NormFinde [19], BestKeeper [20], and the integrated sorting software RefFinder [21] to analyze the stability and variability of potential housekeeping gene for precise data normalization of qRT-PCR results [22].

2. Materials and Methods

2.1. S. chinensis Exogenous Hormone Treatment. The experimental materials came from the *S. chinensis* base of Jilin Agricultural University. Five-year-old *S. chinensis* plants ($n = 45$) with no diseases and insect pests were selected as the experimental material, each of which was a group of one, with three replicates. Water as the control group, four hormones (GA3, IAA, ABA and ZT) were applied via spray bottle to *S. chinensis* plants on July 5 at specific concentrations shown in Table 1. In order to ensure adequate hormone absorption, spraying was then repeated on July 10 and samples were taken on July 20. Collected samples were immediately treated with liquid nitrogen and subsequently stored at -80°C to prevent mRNA degradation. mRNA degradation is an extremely important mechanism for controlling gene expressions in case of bacterial cells. The process includes structured and ordered action of a battery of endonucleases and exonucleases, and various other universal and ones existing are specific species alone.

2.2. Total RNA Isolation and cDNA Synthesis. According to the kit instructions, we extracted RNA from plants using the Spectrum Plant Total RNA Kit (Sigma). Qualified RNA quality was detected by a NanoDrop 2000C Spectrophotometer. Further verification of RNA integrity was conducted using a 1.5% (*w*/*v*) agarose gel electrophoresis. cDNA was synthesized by reverse transcription of RNA with an optical density (OD)260/OD280 ratio between 1.8 and 2.1. The DNA synthesis from an RNA template generates complementary DNA (cDNA). This can work as a template in various downstream applications for conducting RNA studies especially in case of gene expression. Thus, cDNA synthesis acts as the first step for various protocols in molecular biology. The reverse transcriptase (RT) uses the RNA template and complementaries of short primers to the 3′ of RNA to guide the synthesis of the first-strand cDNA. The reverse transcription reaction was performed with 2 μL of total RNA according to the procedure given in the Prime Script™ RT (Takara, Japan) kit. All cDNA samples used in subsequent experiments were stored in a -80°C freezer.

Table 1: The concentration of exogenous hormone spray on *S. chinensis*.

Exogenous hormones	GA_3	IAA	ABA	ZT	H_2O (control group)
Concentration (mg/L)	500	500	500	300	/
	300	300	300	100	/
	100	100	100	50	/

2.3. Candidate Internal Control Gene and Designing of Primers. The qRT-PCR primer pairs for eight reference gene candidates and target gene (GAG) were designed using the Primer 5 software with the following parameters: amplicon sizes were between 140 and 261 bp, primer lengths were between 18 and 22 bp, and amplification efficiencies (E%) between 91 and 109% ranged between linear correlation coefficients (R^2) of 0.982 to 0.998. Indicating that the efficiency of the reaction and degree of matching with the PCR standard curve were good [23, 24], ensuring the accuracy of the qRT-PCR data in subsequent experiments. Primer information is described in Table 2 and Figure 1.

2.4. qRT-PCR Analysis. qRT-PCR assays were performed using the qTOWER 3.0 Thermal Cycler (Analytik Jena, Germany) in 96-well plates. All cDNA samples were thawed and diluted four-fold. The total volume of each reaction was 20 μL, comprised of 10 μL SYBR Premix ExTaq II kit (Takara, Japan), 2 μL dilution cDNA, 1 μL of each primer (forward and reverse), and 6 μL RNase-free water. Following initial denaturation at 95°C for 30 s, 40 amplification cycles were conducted at 95°C for 5 seconds, 57°C for 30 seconds, and 72°C for 30 seconds, respectively. To exclude the formation of primer dimers, a melting curve analysis was performed at a temperature difference of 60°C and 95°C. Three technical replicates were used for each sample, and the same batch of cDNA was used throughout the experiment.

2.5. Data Analysis. Ct values were determined from qRT-PCR for each candidate internal control genes. In addition, the stability analysis of candidate internal control genes was assessed and ranked using four statistical packages: NormFinder, BestKeeper, geNorm, and RefFinder. The NormFinder is an algorithm that is used for identifying the optimal normalization gene from a set of candidate genes. The BestKeeper algorithm helps to identify the best standard genes for normalizing the data. The geNorm algorithm helps in identifying the most stable reference genes from a set of tested candidate reference genes. The RefFinder is a comprehensive tool that is used for evaluating and screening reference genes from large extensive datasets.

2.6. Normalization of GAG. AG belongs to the C functional gene in the ABCD model and plays a central regulatory role in the regulation of the termination of floral meristem development, as well as in the determination of floral organs between stamens and pistils [25, 26]. In the second half of

Table 2: Primer pairs of reference gene and target gene for qRT-PCR in *s. chinensis*.

No.	Genes	Functional description	Gene ID	Primer sequence forward/reverse (5-3)	Length (bp)	E%	R^2
1	*ACTIN*	Actin	TRINITY_DN37950_c0_g1_i1	A:CCATTCCGACCATTACAC S:GATGCCGAGGACATTCAG	142	98	0.995
2	*GAPA*	Glyceraldehyde-3-phosphate dehydrogenase B	TRINITY_DN106361_c0_g1_i1	A:ATCCGTCCAAAGCCGTTA S:CAAGACTCCTCACCCTCA	228	105	0.996
3	*TUBA*	Tubulin alpha-3	TRINITY_DN112332_c0_g2_i1	A:CCGCTCGATGTCAAGGGA S:GGTGGCACTGGGTCTGGTT	237	97	0.996
4	*APX*	Ascorbate peroxidase	TRINITY_DN110618_c1_g1_i1	A:AGCACAGCACCGAAACAT S:ATCTCCTTCCTCGCAACC	170	93	0.995
5	*MUB3*	Membrane-anchored	TRINITY_DN98949_c1_g1_i1	A:GTCCTGCTGTTCTCCAAT S:CCCAGACAAGTATGCTCC	146	103	0.982
6	*UBC4*	Ubiquitin-conjugating enzyme 4	TRINITY_DN161958_c0_g1_i1	A:TGCTTCTATCCGTCTTGTA S:TTCCTCCAGATTATCCATT	231	109	0.994
7	*TIP41*	TIP41-like protein	TRINITY_DN44616_c0_g1_i1	A:CAGCCAACTCGTCTTCATAC S:CAGCAGCCAAGTGGAAAT	261	93	0.991
8	*GAPDH*	Glyceraldehyde-3-phosphate dehydrogenase	TRINITY_DN108444_c3_g8_i3	A: TTTGGCACCACCCTTGAT S:TGTTGGATGGCTCTACTC	170	91	0.998
9	*GAG*	AG flower homologous gene	TRINITY_DN114911_c4_g2_i1	A:GTAATGCCTCGTTCAAGC S:CCACCAAATACAAATCCT	114	92	0.995

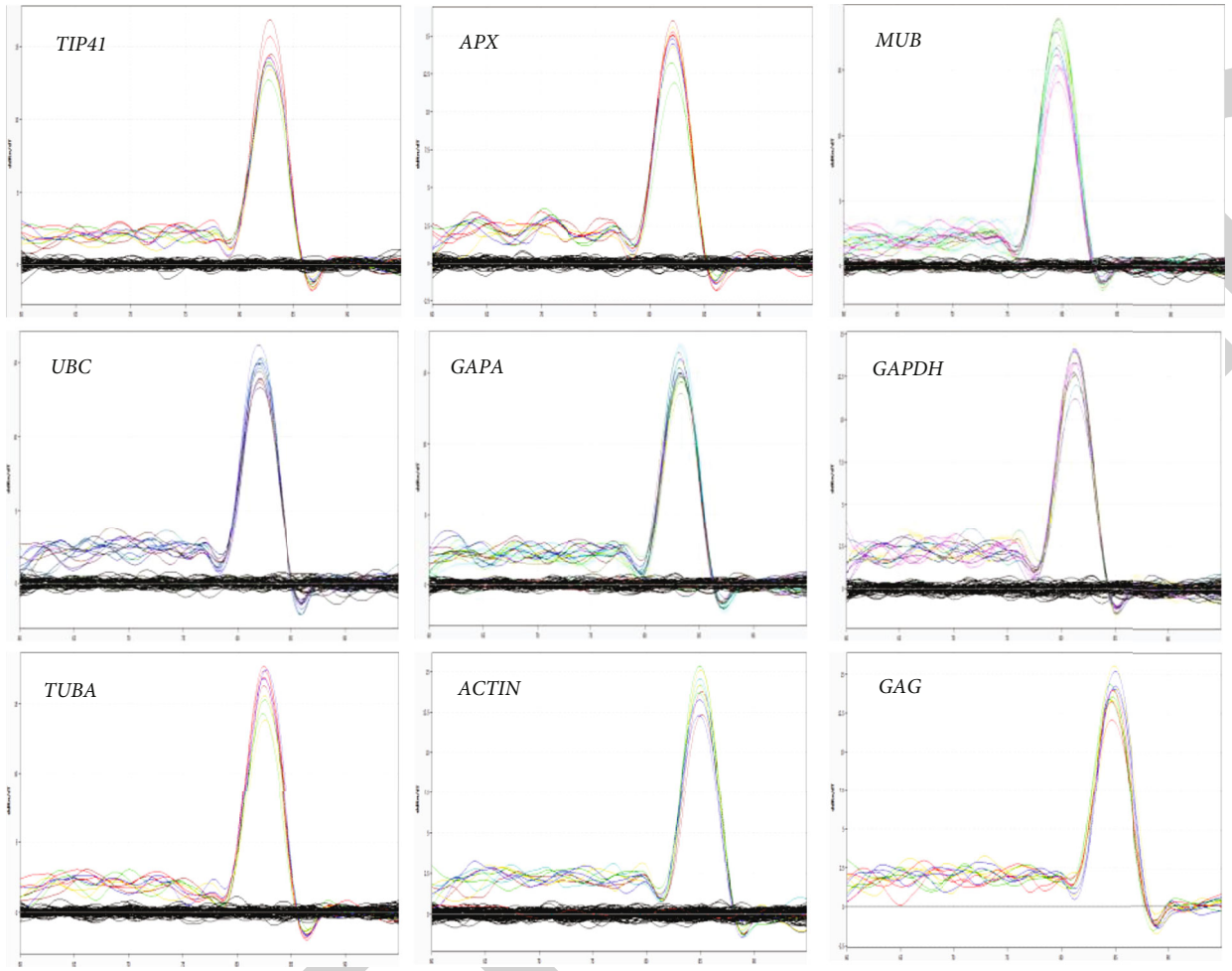

Figure 1: Melt curves generated from each reference gene and target gene (ACTIN, TUBA, MUB, UBC, GAPDH, GAPA, TIP41, APX, and GAG) showing a single peak.

flower ontogeny, it regulates the development of style and carpel and terminates flower development [27]. To further validate the credibility of the housekeeping genes recommended in this work, different combinations of internal control genes were used to detect the expression of GAG gene in female and male flowers.

3. Results

3.1. qRT-PCR Results. Generally, preliminary determination of the abundance of internal control genes can be made using the cycle threshold (Ct) values, in which genes with lower Ct values represent those with higher transcript abundance [28]. Aqualified housekeeping genes should have moderate expression level and above, and its CT value should be $15 < CT < 30$ [29]. In this paper, the eight candidate genes had high or moderate expression levels, with Ct values in the range of 18.44 (ACTIN) to 27.9 (GAPA). Among them, the Ct values of ACTIN, which ranged from 18.44 to 21.44, had the least variation. Conversely, Ct values for GAPA were highly varied, ranging from 22.5 to 27.9 (Figure 2).

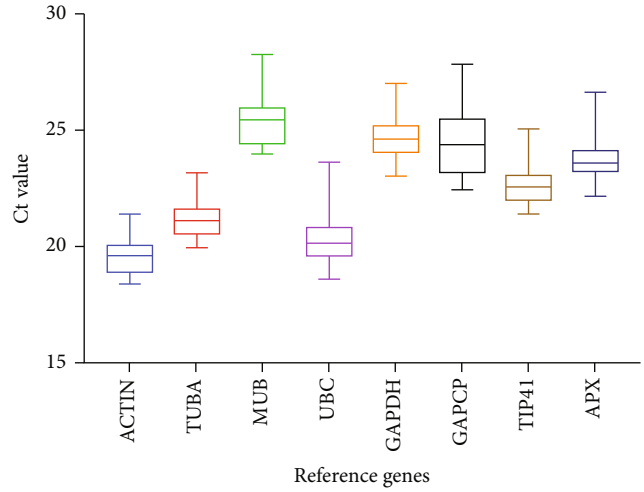

Figure 2: Distribution of crossing point (CT) values for eight internal control genes.

3.2. geNorm Analysis. The geNorm software evaluates the stability ranking and number of internal control genes by calculating the "M value" and the $(Vn/Vn+1)$ value.

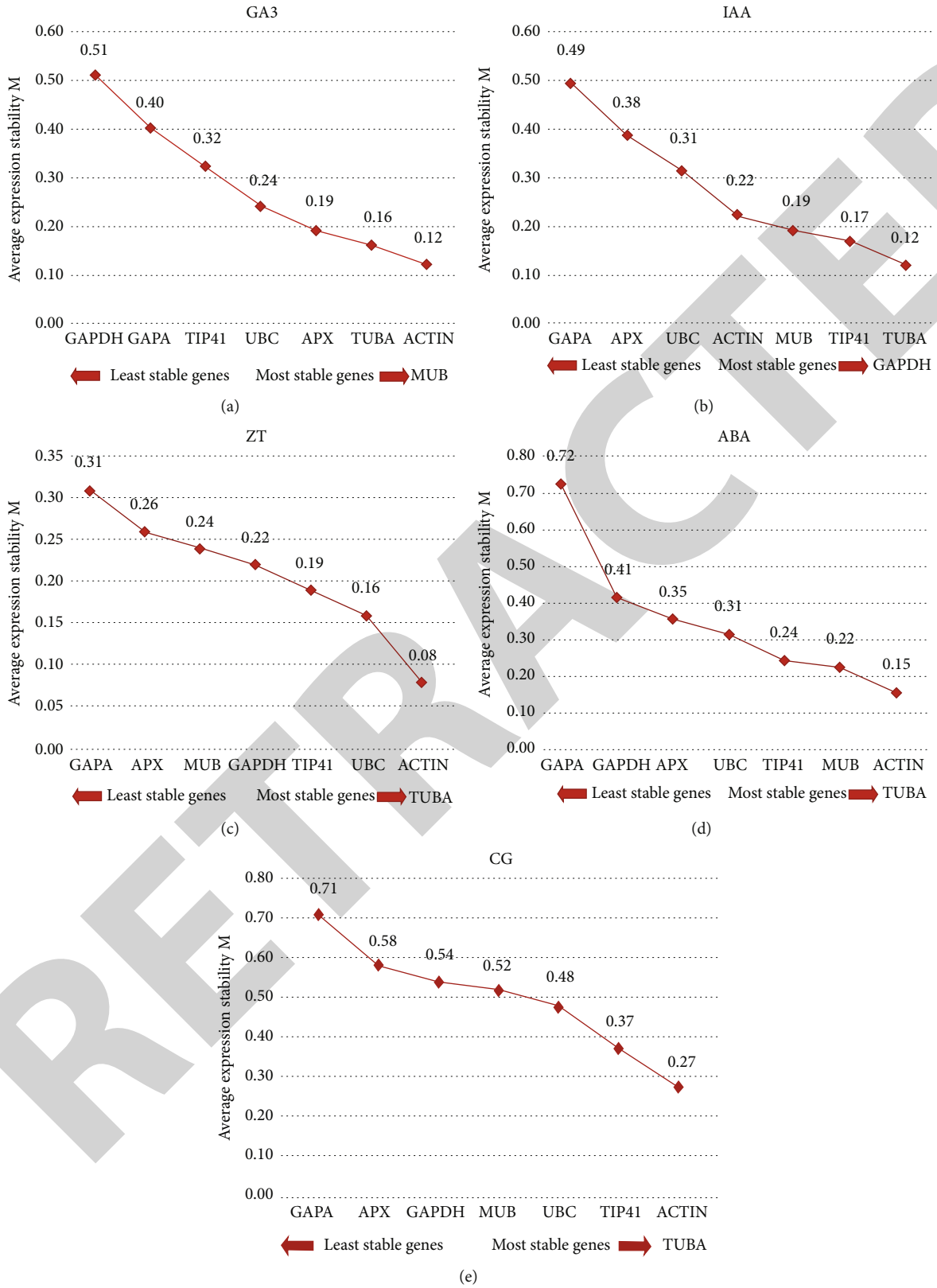

Figure 3: Eight candidate reference gene stability rankings are based on M-values calculated by geNorm. (a) GA3 treatment; (b) IAA treatment; (c) ZT treatment; (d) ABA treatment; (e) control group.

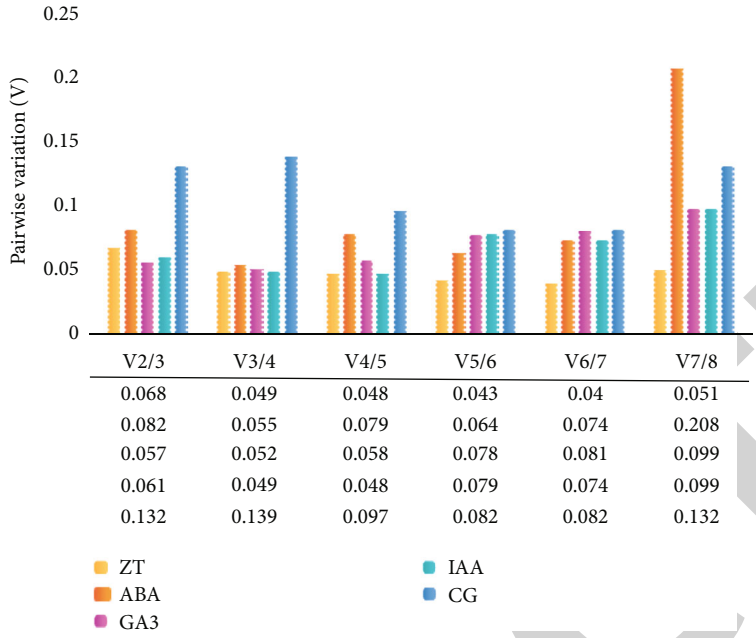

Figure 4: Selection of the optimal number of internal control genes based on the (Vn/n+1) value of geNorm.

Among them, the greater the M value, the worse the stabilization. If the M value of a gene is greater than 1.5, the candidacy of the gene should be cancelled. After geNorm analysis, it was show that the M values of the 8 candidate internal control genes in this paper were all less than 1.5; it was explained that their expression stability met the requirements of experimental analysis (Figure 3). The default threshold for pairwise changes ($Vn/Vn+1$) is 0.15. For calculating variants less than 0.15, select the number of internal control genes (N) suitable for analysis. For calculating pairwise variants greater than 0.15, $N+1$ internal control genes are required. In this paper, the pairwise variation values of all candidate genes in all tissues for all hormone treatment were less than 0.15 (Figure 4), indicating that under all treatment conditions, two internal control genes were sufficient for normalization of gene expression data. The evaluation results of the geNorm software were as follows: The candidate genes ACTIN and MUB had the lowest stable values under GA3 treatment (Figure 3(a)). Under IAA treatment, GAPDH and TUBA were the best possible (Figure 3(b)). And under treatments with ZT or ABA, the optimum composition was ACTIN and TUBA (Figures 3(c) and 3(d)). The M value of ACTIN and TUBA was the smallest and the most stable in the control group (Figure 3(e)).

3.3. NormFinder Analysis. The NormFinder program is computationally similar to geNorm, ranking internal control genes by evaluating their stability values (M) across different experimental designs. The NormFinder tool is used to analyze and asses the stability of expression in case of reference genes involved in qRT-PCR data normalization. The algorithm identifies the optimal normalization gene from a set of candidates. The study in [30] examined six candidate reference genes in leaf, node, internode, root, cabbage, shoot primordial, and basal stem tissues in case of two-year-old clonal oil palms. The NormFinder and BestKeeper algorithms verified the stability of the reference genes in the oil palm tissues being tested. A study used NormFinder and BestKeeper tools for the selection of reliable reference genes while conducting gene expression studies in peach using real-time PCR. The algorithms analyzed the stability of the genes, and it was found that TEF2, UBQ10, and RP II were the optimal reference with high statistical reliability [31]. The study in [11] highlighted the fact that the lowest stability value described genes with the best stability expression level. As shown in Table 3, according to the NormFinder sequence, four internal control genes TUBA (0.053), GAPDH (0.008), TIP41 (0.003), and TUBA (0.019) were calculated to have the lowest stability values under treatment with the four hormones (GA3, IAA, ZT, and ABA). TUBA (0.152) ranked the lowest in control group samples and was considered to the optimum gene candidate.

3.4. BestKeeper Analysis. BestKeeper software ranks the stability of internal control genes mainly in the light of the standard deviation (SD) and coefficient of variation (CV) values of gene expression levels. The algorithm calculates the candidate gene stability considering the standard deviation of Cq values in addition to the coefficient of variance (CV), correlation coefficient and the p-value. It helps in identifying the best standard genes that contribute towards normalization of the data. The lower the two values, the more stable the reference gene. However, genes with a SD value greater than 1 should not be regarded as internal control genes [32]. The results of BestKeeper analysis are shown in Table 4. For hormone treatment (GA3, IAA, ZT, and ABA) and control group samples, the optimal internal control genes were GAPDH (0.22), APX (0.18), TUBA (0.53), ACTIN (0.03), and TIP41 (0.57), respectively, and GAPA was the most unstable gene in all sampling groups.

Table 3: The expression stability of 8 candidate internal control genes under different hormone treatments was evaluated by NormFinder.

No.	GA_3 stress		IAA stress		ZT stress		ABA stress		CG	
	Gene name	M	Gene name	M	Gene name	M	Gene name	M	Gene name	M
1	*TUBA*	0.053	*GAPDH*	0.008	*TIP41*	0.003	*TUBA*	0.019	*TUBA*	0.152
2	*APX*	0.078	*TUBA*	0.032	*ACTIN*	0.107	*MUB*	0.075	*TIP41*	0.239
3	*MUB*	0.089	*MUB*	0.127	*MUB*	0.135	*GAPDH*	0.087	*ACTIN*	0.263
4	*ACTIN*	0.107	*TIP41*	0.129	*TUBA*	0.152	*TIP41*	0.110	*MUB*	0.294
5	*UBC*	0.144	*ACTIN*	0.167	*UBC*	0.154	*ACTIN*	0.194	*UBC*	0.317
6	*TIP41*	0.290	*UBC*	0.307	*GAPDH*	0.155	*UBC*	0.380	*GAPDH*	0.332
7	*GAPA*	0.446	*APX*	0.369	*APX*	0.166	*APX*	0.384	*APX*	0.374
8	*GAPDH*	0.544	*GAPA*	0.542	*GAPA*	0.280	*GAPA*	1.149	*GAPA*	0.721

Table 4: The expression stability of 8 candidate internal control genes under different hormone treatments was evaluated by BestKeeper.

No.	GA_3 stress			IAA stress			ZT stress			ABA stress			CG		
	Gene name	SD	CV	Gene name	SD	CV	Gene name	SD	CV	Gene name	SD	CV	Gene name	SD	CV
1	*GAPDH*	0.22	0.87	*APX*	0.18	0.75	*TUBA*	0.53	2.50	*ACTIN*	0.03	0.14	*TIP41*	0.57	2.39
2	*APX*	0.40	1.71	*ACTIN*	0.32	1.63	*GAPDH*	0.56	2.24	*TUBA*	0.12	0.57	*ACTIN*	0.62	3.08
3	*TIP41*	0.54	2.39	*TIP41*	0.36	1.62	*UBC*	0.56	2.72	*TIP41*	0.16	0.73	*TUBA*	0.67	3.02
4	*TUBA*	0.55	2.65	*GAPDH*	0.45	1.85	*ACTIN*	0.58	3.00	*MUB*	0.26	1.02	*APX*	0.77	3.10
5	*MUB*	0.57	2.31	*TUBA*	0.48	2.30	*TIP41*	0.65	2.90	*UBC*	0.26	1.27	*UBC*	0.78	3.53
6	*ACTIN*	0.58	3.04	*MUB*	0.50	1.98	*MUB*	0.67	2.67	*APX*	0.30	1.29	*GAPDH*	0.81	3.14
7	*UBC*	0.60	3.04	*UBC*	0.71	3.56	*APX*	0.76	3.28	*GAPDH*	0.43	2.00	*MUB*	0.86	3.16
8	*GAPA*	0.98	4.04	*GAPA*	0.86	3.56	*GAPA*	0.96	4.06	*GAPA*	1.50	6.19	*GAPA*	1.02	3.97

Table 5: The expression stability of 8 candidate internal control genes under different hormone treatments was analyzed by RefFinder.

No.	GA_3 stress		IAA stress		ZT stress		ABA stress		CG	
	Gene name	M	Gene name	M	Gene name	M	Gene name	M	Gene name	M
1	*TUBA*	1.86	*GAPDH*	1.41	*TUBA*	1.86	*TUBA*	1.41	*TUBA*	1.32
2	*MUB*	2.34	*TUBA*	2.11	*ACTIN*	2.00	*ACTIN*	2.11	*TIP41*	1.86
3	*APX*	2.63	*TIP41*	3.22	*TIP41*	2.11	*MUB*	2.34	*ACTIN*	2.06
4	*ACTIN*	3.13	*ACTIN*	3.98	*UBC*	3.50	*TIP41*	3.46	*UBC*	4.68
5	*GAPDH*	4.76	*MUB*	4.12	*MUB*	4.56	*UBC*	5.18	*MUB*	5.38
6	*TIP41*	5.05	*APX*	4.30	*GAPDH*	4.82	*GAPDH*	5.21	*GAPDH*	5.48
7	*UBC*	5.44	*UBC*	6.24	*APX*	7.00	*APX*	6.48	*APX*	6.09
8	*GAPA*	7.24	*GAPA*	8.00	*GAPA*	8.00	*GAPA*	8.00	*GAPA*	8.00

3.5. RefFinder Analysis. RefFinder is a web-based online tool that comprehensively ranks the results of geNorm, NormFinder, and BestKeeper assessments to obtain the best stability internal control genes for *S. chinensis* [20]. Based on the RefFinder ranking, TUBA was comprehensively ranked first in all test groups. GAPA was considered to have the worst stability among all candidate genes. Results from RefFinder analysis are provided in Table 5.

3.6. Validation of Reference Gene Selection. According to the RNA sequencing results of female and male flowers of *S. chinensis*, the AG homologous gene GAG was upregulated in female flowers and downregulated in male flowers. The results of the qRT-PCR show that when TIP41, TUBA or TIP41, and TUBA were used as internal control genes, the relative expression of GAG gene in female flower buds was greater than 1, and the expression in male flower bud was less than 1, indicating that GAG gene was upregulated in female flower bud. This result was consistent with the result of RNA-sequencing. Using an inappropriate reference gene can lead to large errors in qRT-PCR results and even erroneous results. Further illustrate the importance of reference genes for RT-qPCR analysis of genes (Figure 5).

4. Discussion

Accurate qRT-PCR results are affected by many kinds of factors including RNA extraction efficiency, reverse

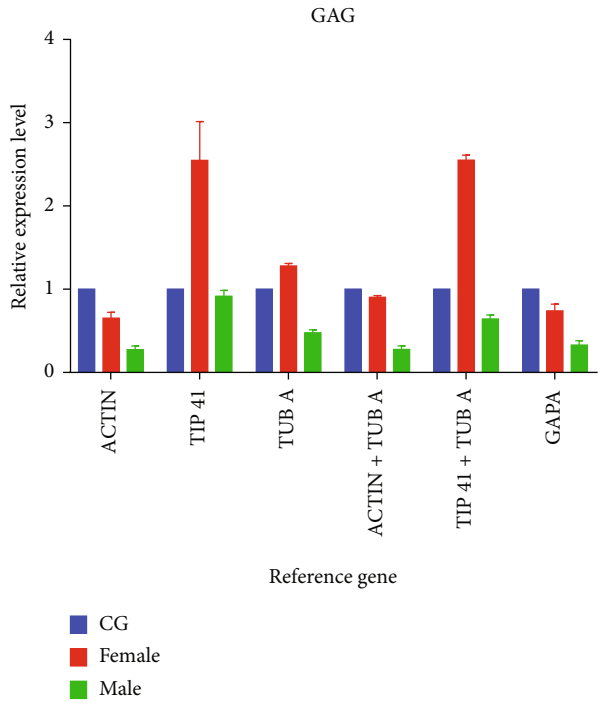

Figure 5: Relative expression of GAG genes when using different internal control genes.

transcriptional, and changes in amplification efficiency [28, 33]. The selection of reasonable internal control genes is the key to improving the accuracy of gene normalization during qRT-PCR detection [34, 35]. In this paper, eight candidate housekeeping genes (GAPDH, ACT, MUB, UBC, TIP41, APX, CPAP, and TUBA) were chosen based on the RNA-sequencing data of male and female flowers of *S. chinensis*. The stability of the genes was ranked by four (4) recognized reference gene evaluation statistical software. The result analysis displayed that the reference genes recommended by geNorm, NormFinder, and BestKeeper were not completely consistent. The appearance of such results may be due to their different calculation rules and analysis procedures [36]. To determine the final ranking of the stabilization of the internal control genes, the results of the algorithm were integrated using the online tool RefFinder, and a comprehensive ranking of the 8 candidate genes was performed. After comprehensive analysis, it was confirmed that TUBA was recommended as the most excellent reference gene for all experimental treatments, and CPAP was the most unstable. TUBA, as the best stability reference gene among *S. chinensis* candidates, was also used as an internal reference gene for celery studies at different developmental stages [23]. But when celery was subjected to four abiotic stresses (drought, cold, high temperature, and salinity) and four hormones (ABA, GA, MeJA, and SA), TUBA became the least stable reference gene [37]. This indicates that even the same internal control genes have different stability under different conditions. However, as qRT-PCR calibration and standardization by a single reference gene may lead to the deviation of the results, it is recommended to use two or more internal control genes to help the accuracy of the results [38]. In all of the samples, the paired variation value (Vn/Vn+1) of the internal control gene standardization factor was less than 0.15, indicating that the two internal control genes could meet the relative quantitative requirements and that it is unnecessary to introduce the third reference gene for correction. Thus, according to the ranking results of RefFinder, in the GA3 treatment group, TUBA and MUB were the optimum combinations; The best partners of the internal control genes in the IAA experimental group were TUBA and GAPDH; TUBA and ACTIN were identified as the best possible housekeeping genes in the ZT and ABA experimental groups; the combination of TUBA and TIP41 exhibited the best stability in the control group. The research shows that the housekeeping genes MUB, ACTIN, TIP41, and GAPDH have different stabilities in different plants or under different experimental settings. For instance, TIP41 was the most reliable internal control gene for *Momordica charantia* under different stress treatments (H_2O_2, NaCl, PEG6000, $CuSO_4$, MeJA, cold, and UV conditions) [8]. F-box and TIP41 were the best stability combination of internal control genes under drought stress in *Hedera helix* L. [39], but TIP41 was not the best reference gene in three tree peony cultivars [28]. Other housekeeping genes have similar conclusions. The stabilization of the same reference gene in different plants and different experimental treatments is also different [11, 32–42]. By using different internal control genes to detect the expression of GAG gene in male and female flowers, the reliability of the recommended internal control genes in this work was further verified. In summary, normalization of different internal control genes affects the accuracy of qRT-PCR data; that is, unstable internal control genes may lead to significant deviations from true expression levels and to a misunderstanding of data [3, 33]. Therefore, it is the key to evaluate the reliability and reproducibility of reference gene expression and a crucial choice for the appropriate internal control genes according to the research conditions.

5. Conclusions

To sum up, this work evaluated and ranked the stability of 8 candidate internal control genes in four hormones through four evaluation software and further verified the reliability of this study through GAG gene. The result proves that these housekeeping genes had different stability under different experimental conditions. When one reference gene was used, selecting TUBA satisfies all experimental groups. When two internal control genes are used, TUBA and MUB can be used in the GA3 group; the combination of TUBA and GAPDH can be selected in the IAA treatment group; TUBA and ACTIN are available for ZT and ABA experimental groups; TUBA and TIP41 are recommended as the most adaptive internal control genes for the control group; GAPA is the most unreliable as an internal reference gene for each experimental group of *S. chinensis*. This paper thus identifies suitable internal control genes for the expression study of *S. chinensis* under different hormone treatments.

Data Availability

The datasets used during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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