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Reference gene selection for qRT-PCR analysis of flower development in *Lagerstroemia indica* and *L. speciosa*

Tangchun Zheng[1,2,3,4☯], Zhilin Chen[1,2,3,4,5☯], Yiqian Ju[1,2,3,4,5], Han Zhang[1,2,3,4,5], Ming Cai[1,2,3,4,5], Huitang Pan[1,2,3,4,5], Qixiang Zhang[1,2,3,4,5]*

1 Beijing Key Laboratory of Ornamental Plants Germplasm Innovation & Molecular Breeding, Beijing Forestry University, Beijing, China, **2** National Engineering Research Center for Floriculture, Beijing Forestry University, Beijing, China, **3** Beijing Laboratory of Urban and Rural Ecological Environment, Beijing Forestry University, Beijing, China, **4** Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants of Ministry of Education, Beijing Forestry University, Beijing, China, **5** School of Landscape Architecture, Beijing Forestry University, Beijing, China

☯ These authors contributed equally to this work.
* zqxbjfu@126.com

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Abstract

Quantitative real-time polymerase chain reaction (qRT-PCR) is a prevalent method for gene expression analysis, depending on the stability of the reference genes for data normalization. *Lagerstroemia indica* and *L. speciosa* are popular ornamental plants which are famous for the long flowering period. However, no systematic studies on reference genes in *Lagerstroemia* have yet been conducted. In the present study, we selected nine candidate reference genes (*GAPDH*, *TUA*, *TUB*, *18S*, *RPII*, *EF-1α*, *ATC*, *EIF5A* and *CYP*) and evaluated their expression stability in different tissues during floral development of *L. indica* and *L. speciosa* using four algorithms (geNorm, NormFinder, BestKeeper and, RefFinder). Results showed that *RPII* and *EF-1α* were the most stably expressed and suitable reference genes for both of *Lagerstroemia* species. Moreover, *ACT* exhibited high expression stability in *L. indica* and *GAPDH* was a suitable reference gene for *L. speciosa* in different flower development stages. *TUB* was an unsuitable reference gene for gene expression normalization due to significant variations in expression across all samples. Finally, we verified the reliability of the selected candidate reference genes by amplifying an *AGAMOUS* homolog (*LsAG1*) of *Arabidopsis thaliana*. This study provides a list of suitable reference genes, thereby broadening the genetic basis of the gene expression patterns in *Lagerstroemia* species.

Introduction

qRT-PCR is a popular method to study gene expression with high sensitivity, specificity and adaptability to high throughput analyses [1–3]. It bases on the use of reference gene as the normalization of gene transcription levels requires the stability of the selected reference genes [4,5]. Traditional reference genes, including *18S rRNA* (*18S ribosomal RNA*), *GAPDH* (*glyceraldehyde-3-phosphate dehydrogenase*), *EF-1α* (*elongation factor-1-alpha*), *TUA* (*alpha-tubulin*),