Supplementary Information

Title of Manuscript: Selection of Reference Genes for qPCR Analyses of Gene Expression in Ramie Leaves and Roots across Eleven Abiotic/Biotic Treatments

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This Supplementary File contains the following information:

1. Figure S1. Melting curves of the eight candidate reference genes among biological and technical replicates.
2. Table S1. Digital expression analysis of candidate reference genes in ramie under various stresses.
3. Table S2. Information of eight candidate reference genes.
4. Table S3. Ranking the candidate reference genes across leaves and roots of ramie under different treatments as determined via RefFinder.

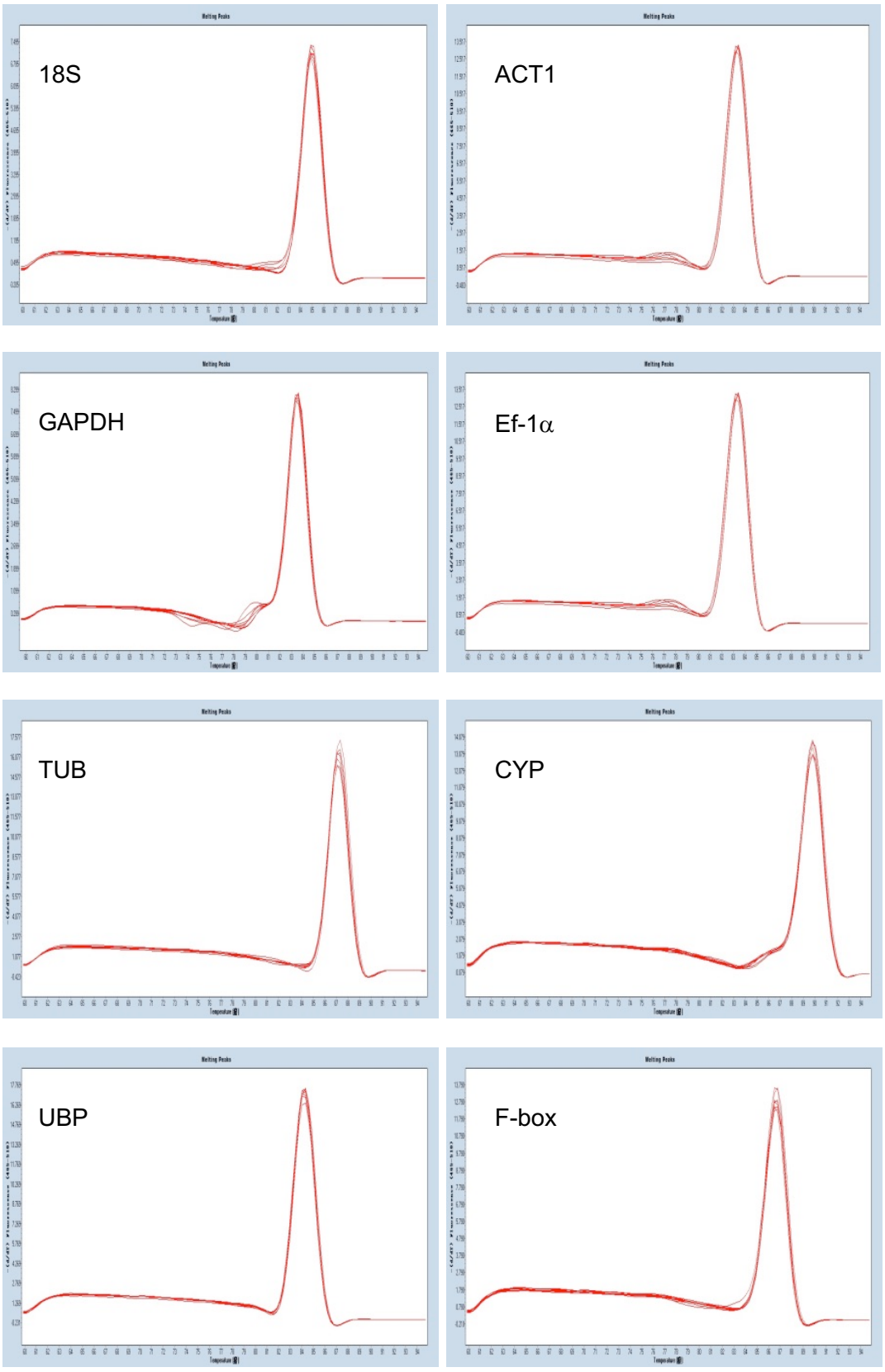

Figure S1. Melting curves of the eight candidate reference genes in ramie.

Table S1 Digital expression analysis of candidate reference genes in ramie under various stresses

Gene Symbol	Gene name	Regulation under Nematode infection [1]	Regulation under Nematode infection [2]	Regulation under Cd stress [3]	Regulation under drought stress [4]	Regulation under ramie moth infestation [5]
18S	18S rRNA (AF206870.1)	none	up	none	none	none
ACT1	Actin1 (DQ665832.2)	none	none	none	none	none
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	none	none	none	none	down
EF-1α	Elongation factor 1-alpha	none	none	none	none	none
αTUB	a-tubulin	none	none	none	none	none
CYP	Cyclophilin2	none	none	none	none	none
UBQ	Ubiquitin	none	none	none	none	none
F-box	F-box family	none	none	none	none	none

Note: RNA-Seq based identification of differentially expressed genes in: stems and leaves (1, Zhu et al. 2014), roots (2, Yu et al. 2015) of ramie challenged by Pratylenchus coffeae, whole plants of ramie under CdCl2 stress (3, Liu et al. 2015), leaves and roots of ramie under draught stress (4, An et al. 2015), leaves of ramie infested by ramie moth larvae (5, Zeng et al. 2106).

References

1. Zhu, S., Tang, S., Tang, Q., & Liu, T. (2014). Genome-wide transcriptional changes of ramie (Boehmeria nivea L. Gaud) in response to root-lesion nematode infection. Gene, 552(1), 67-74.

2. Yu, Y., Zeng, L., Yan, Z., Liu, T., Sun, K., Zhu, T., & Zhu, A. (2015). Identification of ramie genes in response to Pratylenchus coffeae infection challenge by digital gene expression analysis. International journal of molecular sciences, 16(9), 21989-22007.

3.Liu, T., Zhu, S., Tang, Q., & Tang, S. (2015). Genome-wide transcriptomic profiling of ramie (Boehmeria nivea L. Gaud) in response to cadmium stress. Gene, 558(1), 131-137.

4. An, X., Chen, J., Zhang, J., Liao, Y., Dai, L., Wang, B., ... & Peng, D. (2015). Transcriptome profiling and identification of transcription factors in ramie (Boehmeria nivea L. Gaud) in response to PEG treatment, using illumina paired-end sequencing technology. International journal of molecular sciences, 16(2), 3493-3511.

5. Zeng, L., Shen, A., Chen, J., Yan, Z., Liu, T., Xue, Z., & Yu, Y. (2016). Transcriptome analysis of ramie (Boehmeria nivea L. Gaud.) in response to ramie moth (Cocytodes coerulea Guenée) infestation. BioMed research international, 2016.

Table S2 Information of eight candidate reference genes.

Gene symbol	Gene description	Gene ID	Homolog ID	Gene expression	*E* score	Sequence identity (%)	Reference
18S	18S rRNA	AF206870.1	JF317363.1	Debregeasia saeneb 18S ribosomal RNA gene	0	99	Zhang et al. 2011
ACT1	Actin1	DQ665832.2	HQ163776	Morus alba actin 1 (ACT1) mRNA, complete cds	0	92	Li et al. 2011
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	comp37700_c0	XM_010093908	Morus notabilis NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (LOC21386841), mRNA	0	88	He et al. 2013
*EF-1*α	Elongation factor 1-alpha	comp36076_c0	JN399225.1	Rosa multiflora elongation factor 1-alpha mRNA, complete cds	0	91	Klie & Debener. 2011
αTUB	alpha-tubulin	comp24081_c0	X67162	P.amygdalus mRNA for alpha-tubulin	3E-97	97	Stocker et al. 1993
CYP2	Cyclophilin2	comp26478_c0	NM_001357079	Glycine max peptidyl-prolyl cis-trans isomerase CYP2 (GMCYP2), mRNA	2E-180	90	Mainali et al. 2014
UBQ	Ubiquitin	comp20072_c0	XM_016024811	Ziziphus jujuba ubiquitin-60S ribosomal protein L40 (LOC107416326), mRNA	4E-101	88	Liu et al. 2014
F-box	F-box family	comp30228_c0	XM_024174059	Morus notabilis putative F-box protein PP2-B12 (LOC21389887), mRNA	3E-127	77	He et al.2013

Table S3. Ranking the candidate reference genes across leaves and roots of ramie under different treatments as determined via RefFinder.

Rank	Total	Abiotic	Hormone	*P. vexans*	SA	BTH	JA	ETH	GA3	Heat	Cold	Na	Cd	PEG
1	ACT1 (1.19)	ACT1 (1.57)	GAPDH (2.00)	F-box (1.00)	F-box (1.78)	F-box (1.68)	18S (1.50)	F-box (1.78)	18S (1.41)	18S (1.63)	F-box (1.68)	ACT1 (1.19)	ACT1 (1.19)	EF-1a (1.73)
2	GAPDH (2.45)	UBQ (2.34)	TUB (2.45)	EF-1a (1.86)	TUB (2.06)	18S (1.97)	GAPDH (2.21)	GAPDH (2.91)	F-box (1.86)	TUB (2.21)	CYP2 (2.06)	F-box (2.63)	F-box (2.63)	GAPDH (2.11)
3	TUB (2.83)	F-box (2.55)	ACT1 (3.13)	UBQ (2.71)	EF-1a (2.21)	EF-1a (2.45)	ACT1 (2.28)	TUB (3.13)	GAPDH (3.66)	UBQ (3.08)	UBQ (2.51)	EF-1a (2.99)	EF-1a (2.99)	ACT1 (2.45)
4	F-box (3.98)	EF-1a (3.94)	CYP2 (3.87)	18S (4.00)	18S (4.40)	ACT1 (3.34)	TUB (3.66)	UBQ (3.64)	ACT1 (3.98)	F-box (3.83)	TUB (2.94)	CYP2 (4.05)	CYP2 (4.05)	F-box (2.99)
5	18S (4.76)	18S (4.28)	F-box (3.98)	ACT1 (5.23)	GAPDH (4.43)	CYP2 (4.43)	EF-1a (4.47)	EF-1a (3.66)	EF-1a (4.12)	ACT1 (3.94)	ACT1 (4.68)	TUB (4.76)	TUB (4.76)	18S (3.76)
6	UBQ (5.48)	GAPDH (4.47)	18S (4.33)	GAPDH (6.19)	ACT1 (4.76)	TUB (5.63)	F-box (4.90)	CYP2 (4.45)	UBQ (5.44)	CYP2 (4.16)	18S (6.24)	GAPDH (4.79)	GAPDH (4.79)	TUB (6.00)
7	EF-1a (6.19)	TUB (6.24)	EF-1a (4.45)	TUB (6.70)	CYP2 (6.45)	GAPDH (6.48)	UBQ (7.00)	18S (5.24)	TUB (5.66)	GAPDH (6.09)	GAPDH (6.26)	18S (5.42)	18S (5.42)	UBQ (7.00)
8	CYP2 (6.40)	CYP2 (7.74)	UBQ (7.44)	CYP2 (7.74)	UBQ (7.00)	UBQ (7.74)	CYP2 (8.00)	ACT1 (6.73)	CYP2 (6.96)	EF-1a (8.00)	EF-1a (7.24)	UBQ (7.24)	UBQ (7.24)	CYP2 (8.00)