Appendix A. supplementary data

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"Evaluation of reference genes for quantitative real-time PCR normalization in cotton bollworm, Helicoverpa" (*Molecular Biology, 2014, Vol. 48, No* 6)

Fig. S1: Representative melt-curve profiles of RGs and target gene in Real-Time PCR assay.

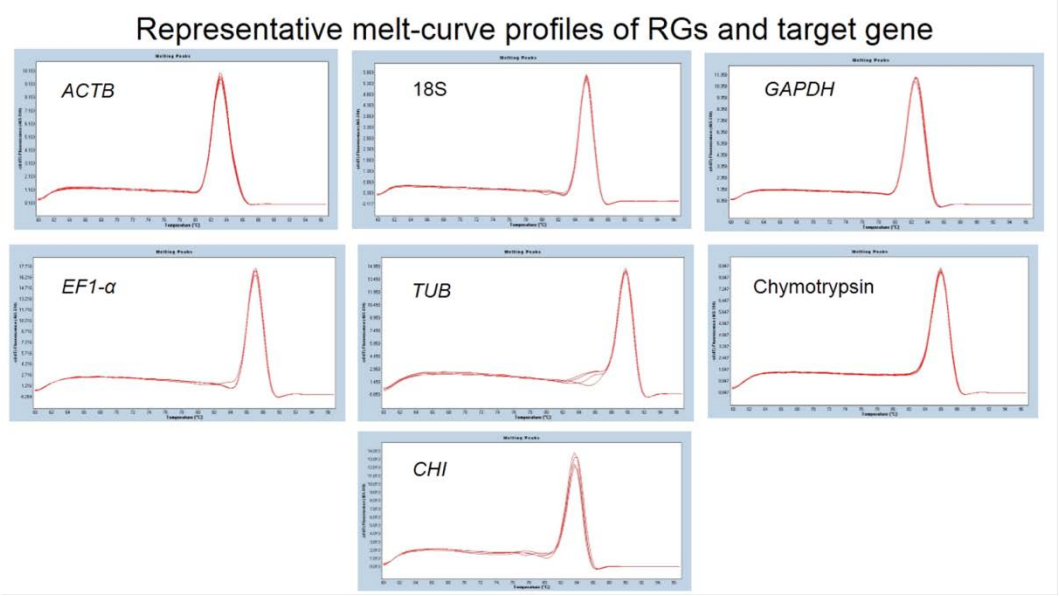

Table S1: Description of gene-specific primers used in this study

Name of the Gene	Symbol	GenBank Accession No	Primer Sequence (5'->3')	Tm (°C)	Amplicon Size (bp)	Function
Primers used for Real-Time PCR assays						
F: GCCCAGAGCAAGAGAGGTAT	61.9	Structural constituent of cytoskeleton	100	β-actin	*ACTB*	HM629436.1
R: AGAAGGTGTGGTGCCAGAT			62			
F: CACACCACTAGAGGACACAGA	62.1	Structural constituent of ribosome	95	18S rRNA	*18S*	AJ577253.1
R: GAACACATAACGACGGACGAG			61.8			
F: AGGCTGGTGCTGAATACG	60.8	JF417983.1	103	Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity	*GAPDH*	Glyceraldehyde-3-phosphate dehydrogenase
R: GCGGAGATGATGACCTTCTT			60.9			
F: CTCGTCCCACAGACAAGG	60.6	U20129.1	Translation elongation factor activity; GTPase activity; GTP binding	*EF1-α*	Elongation fator-1 alfa	111
R: TACCAGGCTTCAGGATACCA			61.5			
F: TCGTCATACTTCGTGGAATGGAT	62.9	JF767013.1	Cytoskeletal structure protein	*TUB*	β-tubulin	114
R: CTGGATGGCGGTGGAGTT			63.1			
F: CAGGTGGTCAGGCTGTCT	61.8	HM209422.1	CHY	135	Digestive serine proteases	Chymotrypsin
R: CGGAGAACAAAGTGGTGGAA			61			
F: TGCGGTTGGTAATTGTTTTCTC	61.1	chalcone isomerase	109	Flavonols biosynthesis	CHI	NM_001247492.1
R: TCCTCATTCTTTCCACCTGTAAG			61.3			
Primers used for dsRNA synthesis						
F: *TAATACGACTCACTATAGGG*CTAGCAGGATCGTCGGTGGT	73.3	463	Digestive serine proteases	Chymotrypsin	CHY	HM209422.1

R: TAATACGACTCACTATAGGGGATCACTGGCAGGTTGACGT	72.8
Note: F: Forward primer; R: Reverse primer; Italicised letters are T7 Promoter sequence	

Table S2: MIQE guidelines checklist

Item Check	Importance	
Experimetal Design		
Definition of experimental and control groups	E	**Experimental groups**: **a.** dsRNA treatment: Larvae treated with chymotrypsin dsRNA. Control groups:larvae without treatment, **b:** Insect developmental stgaes mentioned in MS.
Number within each group	E	n=20
Assay carried out by the core or investigator's laboratory	D	
Acknowledgment of authors' contributions?	D	
Sample		
Description	E	**Experimental groups**: **a.** dsRNA treatment: Larvae treated with chymotrypsin dsRNA. Control groups:larvae without treatment, **b:** Insect developmental stgaes mentioned in MS.
Volume/mass of sample processed	D	20 mg
Microdissection or macrodissection	E	Total insect
Processing procedure	E	Insect immobilized by immersing in chloroform
If frozen, how and how quickly?	E	Immediately used
If fixed, with what and how quickly?	E	Not applicable
Sample storage conditions and duration	E	Not applicable
Nucleic acid extraction		
Procedure and/or instrumentation	E	Total RNA was extracted using the ISOLATE RNA Mini Kit (Bioline Reagents Ltd., UK) following manufacture's protocol. 20 mg of Larval tissue freezed and ground in mortar using pistel. Lysis to extraction was performed exactly according manufacturer's instructions.
Name of kit and details of any modifications	E	ISOLATE RNA Mini Kit (Bioline Reagents Ltd., UK)
Source of additional reagents used	D	Not applicable