J Biosci Vol. 43, No. 1, March 2018, pp. 173–187
DOI: 10.1007/s12038-017-9728-z

Review

Plant reference genes for development and stress response studies

JOYOUS T JOSEPH, NAJYA JABEEN POOLAKKALODY and JASMINE M SHAH*
Department of Plant Science, Central University of Kerala, Padannakkad, Kasaragod 671 314, India

*Corresponding author (Email, jasmine@cukerala.ac.in, jas109@gmail.com)

MS received 21 July 2017; accepted 18 December 2017; published online 9 February 2018

Many reference genes are used by different laboratories for gene expression analyses to indicate the relative amount of input RNA/DNA in the experiment. These reference genes are supposed to show least variation among the treatments and with the control sets in a given experiment. However, expression of reference genes varies significantly from one set of experiment to the other. Thus, selection of reference genes depends on the experimental conditions. Sometimes the average expression of two or three reference genes is taken as standard. This review consolidated the details of about 120 genes attempted for normalization during comparative expression analysis in 16 different plants. Plant species included in this review are Arabidopsis thaliana, cotton (Gossypium hirsutum), tobacco (Nicotiana benthamiana and N. tabacum), soybean (Glycine max), rice (Oryza sativa), blueberry (Vaccinium corymbosum), tomato (Solanum lycopersicum), wheat (Triticum aestivum), potato (Solanum tuberosum), sugar cane (Saccharum sp.), carrot (Daucus carota), coffee (Coffea arabica), cucumber (Cucumis sativus), kiwi (Actinidia deliciosa) and grape (Vitis vinifera). The list includes model and cultivated crop plants from both monocot and dicot classes. We have categorized plant-wise the reference genes that have been used for expression analyses in any or all of the four different conditions such as biotic stress, abiotic stress, developmental stages and various organs and tissues, reported till date. This review serves as a guide during the reference gene hunt for gene expression analysis studies.

Keywords. Biotic and abiotic stress; normalization; plant; real-time PCR; reference genes; stable expression

1. Introduction

Comparative gene expression analysis requires one or more reference genes to reflect the amount of RNA or cDNA transcribed under a particular condition or treatment in an experiment. While analysing two samples of different experimental conditions, or tissues, expression of the reference gene is taken as internal standard. Formerly, relative quantification of transcription was done by Northern blot analysis or by reverse transcription polymerase chain reaction (RT-PCR). Northern blot often used the amount RNA loaded as a reference. The problem associated with RNA was that the visible band was of rRNA and not mRNA (Shah et al. 2009). So several laboratories re-probed the Northern blots with cDNA of reference genes like housekeeping genes (HKG) (Dean et al. 2002). Such reference genes were amplified for comparison of RT-PCR results as well. The images of Northern blots and RT-PCR gels were captured using digital cameras and the information was processed by software (example, NIH Image program), which normalized the band intensity of gene of interest with that of reference gene and calculated the fold change (Dean et al. 2002). The limitations of this method were that the calculation was not precise, was less sensitive, and could not detect extremely low expression. Soon, Northern hybridization and RT-PCR evolved into microarray and real-time PCR, respectively. While the former could analyse thousands of genes at a time, only one gene could be analysed in the later method. One requirement which was inevitable all throughout this evolution was that of an appropriate reference gene to normalise the RNA levels.

Microarray and real-time PCR techniques are highly sensitive and can calculate the precise fold change during a comparative expression analysis. This precision is highly dependent on the expression of the reference gene. It is most important that the reference gene exhibited stable expression during various treatments considered for comparison. There are reports of about 120 genes, analysed for their potential to be used as reference genes in plants subjected to various treatments. In this review we have consolidated details of these genes, the nature of stress/treatment and, the stability of these genes as well.

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12038-017-9728-z) contains supplementary material, which is available to authorized users.

2. Need for reference genes

Reference genes are required in experiments that involve comparison of target gene expression due to variation in factors like stress (biotic or abiotic), developmental stage, treatment, etc. Target gene expression analyses involves preparation of mRNA, cDNA synthesis, followed by real-time PCR or microarray. Each of these steps are prone to variation in sample preparation, quantification, handling, etc. (Pfaffl 2004; Guénin et al. 2009). To overcome this variation, target gene mRNA levels are normalized to reference gene mRNA level in the reaction after reverse transcribing it into cDNA (Bustin 2002). Since there are many reviews on the critical assessment of what defines a suitable reference gene and how such genes would be thoroughly verified (Huggett et al. 2005; Guénin et al. 2009; Dundas and Ling 2012; Kozera and Rapacz 2013; Rocha et al. 2015; Chapman and Waldenström 2015), such details are not elaborated in this review. The expression of reference gene is expected to be unaffected in the given condition. Hence, the challenge for any expression analysis involves identifying a suitable reference gene for that condition.

3. Nominees for reference genes

Any gene that has stable expression in the given condition can be used as a reference gene. Most commonly used internal controls are housekeeping genes (HKGs), due to their ability to express in all cells of an organism despite normal as well as patho-physiological conditions. HKGs are constitutive genes required for the maintenance of basic cellular functions like cell division, growth and development, apoptosis and, physiological processes such as glycolysis, metabolism, etc. Some of the most widely and traditionally used HKGs that are used as internal control include Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Elongation factor-1α (EF-1 α), Polyubiquitin (UBQ), Actin, α-Tubulin, β-Tubulin, 18S rRNA, 25S rRNA, Ubiquitin-conjugating enzyme E2 (UBC), Eukaryotic initiation factor 1 (EIF1), Eukaryotic transcription factors, etc., (Kozera and Rapacz 2013). In addition to HKGs, genes unrelated to housekeeping activity, with even unknown functions, can also be used for normalization purpose by virtue of their stable expression (Czechowski 2005).

4. The instability of reference genes

Stability of the reference gene expression can be influenced by abiotic, biotic, and developmental factors affecting the cells in which they express. Hence, reference gene for one experiment may be unfit for another experiment; even if the same experimental organism is used. For instance, Actin, which is routinely used as a reference gene for studying gene expression under biotic as well as abiotic stress in Arabidopsis (Atkinson et al. 2013; Xin et al. 2013; Lacroix and Citovsky 2014; Wu et al. 2014a; Wu et al. 2014b), was least stable under different developmental stages of the plant and, UBQ10 appeared to be the most stably expressed (Czechowski 2005).

Housekeeping gene expression may even vary between plants belonging to same genera as well. For example, under abiotic stress, β-Tubulin exhibited stable expression in Solanum lycopersicum (Løvdal and Lillo 2009), and was unstable in S. tuberosum (Nicot et al. 2005).

Even though the expression level varies upon different stress conditions and developmental stages, one would anticipate a stable expression in all the vegetative parts of the plant under a particular condition. But this presumption could be wrong since some of the housekeeping genes showed variability in different organs of a plant as well. For example, in cotton, Actin14, Histone H3 (HIS3) and Translation elongation factor 1A-8 (EF1A8) genes showed stable expression only in leaves under salt stress whereas α-Tubulin10 and UBQ7 genes stably expressed only in roots (Wang et al. 2013).

Housekeeping genes, even though ubiquitous in all stages of development, may exhibit varied expression level in each stage. It is interesting that, the expression sometimes could vary even with short time interval, such as six-leaved stage and seven-leaved stage, bud sizes from 1 to 8 mm, etc., as observed in Solanum lycopersicum (Expósito-rodríguez et al. 2008). Housekeeping genes may also show uniform expression patterns in different organs of the same plant, but not in different developmental stage, making the selection process even more uncertain. For example, tomato α-Tubulin gene stably expressed in leaf, root, fruit, and flower of the same plant (Coker and Davies 2003), whereas its expression was found to be unstable under different developmental stages of leaf, root, fruit and flower (Expósito-rodríguez et al. 2008).

5. Reference gene identification, an inevitable task

For the expression analysis of any gene under a new treatment condition, the procedure is to ‘start from scratch’, i.e. identify appropriate reference gene, which exhibits stable expression, for that treatment. The expression levels of target genes would be inaccurate and altered transcriptional profiles would be displayed upon normalization if the chosen reference gene is unstable (Løvdal and Lillo 2009; Petriccione et al. 2015). Selection of a stable gene as a reference is an extremely critical step because careless selection can lead to misleading results. False interpretation may arise from false selection (Gutierrez et al. 2008; Guénin et al. 2009).

Since the transcript level is sensitive to parameters like variation in plants, organs, stress condition, developmental stages, etc. selection of an appropriate reference gene often

Table 1. continued

	Expressed (AT4G26410)	*A .tumefaciens* different strains (Ach5, LBA4002, LBA4404, LBA4404(pCAMBIA2300)	Unstable	Joseph *et al.* Unpublished
	EF-1α (AT5G60390)			
	UBC (AT4G27960)			
	AP2M(AT5G46630)			
	Actin 2(AT3G18780)			
	β-Tubulin 8 (AT5G23860)			
	Tubulin α-4 (AT1G04820)			
	Tubulin α-5 (AT5G19780)			
	UBC28 (AT1G64230)			
	Unknown protein (AT4G26410)			
	SAND (AT2g28390)	*Phytophthora infestans, Albugo laibachii*	Stable	Belhaj *et al.* 2016
	PPC2 (At2g42600)	*Erysiphe cichoracearum*		Fauteux *et al.* 2006
	Developmental stages			
	CACS (AT5G46630)	Developmental series (79 different tissues, organs, developmental stages, and genotypes)	Stable	Czechowski *et al.* 2005
	Helicase (At1g58050)			
	Expressed (AT4G26410)			
	TIP41-like (AT4G34270)			
	Actin 2 (AT3G18780)		Unstable	
	Tubulin 6 (At5g12250)			
	Actin 7 (At5g09810)			
	CBP 20 (At5g44200)			
	CACS			
Nicotiana benthamiana	**Abiotic stress**			
	PP2A (TC21939)	*Tobacco necrosis virus A, Beet black scorch virus, Beet necrotic yellow vein virus, Barley stripe mosaic virus and Potato virus X*	Stable	Liu *et al.* 2012
	F-BOX			
	60S RPL23 (TC19271)			
	GAPDH (TC21175)		Unstable	
	eEF1-α	*Pseudomonas syringae* pv. tomato	Stable	Catinot *et al.* 2008
	Biotic stress			
	β-Actin	Dehydration	Stable	Archana *et al.* 2009
	eEF1-α	UVC irradiation		Catinot *et al.* 2008
Nicotiana tabacum	**Organs**			
	PP2A (X97913)	Leaf, stem, root, anther and carpel	Stable	Schmidt and Delaney 2010
	Actin (X69885)			
	α-Tubulin (AJ421411)			
	β-Tubulin (U91564)		Unstable	
	EF-1a	Leaf, stem, root and flower	Stable	Guo *et al.* 2016
	Zn finger (XM_009612124)			
	NADH-DH			
	CPN60-2			
	Type III sec			
	Hypothetical protein			
	GDH		Unstable	
	Zn finger (gi|158189353)			

Table 1. continued

Oryza sativa	**Abiotic stress**			
	eEF1-α (AK061464)	Hormone treatments, salt, drought	Stable	Jain *et al.* 2006
	UBQ5 (AK061988)			
	25srRNA (AK119809)			
	18SrRNA (AK059783)			
	UBC (AK059694)			
	Actin 2 (AK100267)		Unstable	
	β-tubulin (AK072502)			
	GAPDH (AK064960)			
	UBQ10 (AK101547)			
	Actin	NaCl, ABA, JA,SA, Probenazole	Stable	Hashimoto *et al.* 2004
	α- Tubulin	NaCl, cold, heat, dehydration, ABA, pH and light stress		Trivedi *et al.* 2013
	UBQ1	Temperature, salinity, ABA, BTH, defense signaling compounds,		Ueno *et al.* 2015; Chen *et al.* 2006
	Biotic stress			
	UBQ1	*Magnaporthe oryzae*	Stable	Ueno *et al.* 2015
	Actin	*Pyricularia grisea*		Hashimoto *et al.* 2004
	Developmental stages			
	eEF1-α	Seedling, different stages of shoot, root, leaf, rachis, inflorescence, flower and seeds	Stable	Jain *et al.* 2006
	UBQ5			
	eIF-4a (AK073620)			
	UBQ10			
	18SrRNA			
	β-tubulin		Unstable	
Vaccinium corymbosum	**Organs**			
	UBQ3b (CV091027)	Mature fruit, immature fruit, leaf, stem, flower bud, flower, fruit abscission zone, branch abscission zone	Stable	Vashisth and Klima 2011
	UBC28 (CF811189)			
	RH8 (DR067965)			
	CACSa (DR067098)			
	EF-1α (CV090683)		Unstable	
	UBQ3a (CV091371)			
	Actin7(CF811156)			
	RHFP (CV090795)			
	β-tubulin (CV090295)			
	GAPDH (CV091251)			
	PP2A (DR067299)			
	CACSb (CV091378)			
	Abiotic stress			
	Metallothionein	Aluminium treatment, cold treatment	Stable	Blancheteau *et al.* 2011; Naik et al, 2007
	GAPDH	Hormone treatment		Zhang *et al.* 2016
	Actin	Heat, dehydration, salt		Chen *et al.* 2017
	Developmental stages			
	UBC28	Fruits at different stages	Stable	Li *et al.* 2016

Table 1. continued

	Abiotic stress				
Gossypium hirsutum	*HIS3 (AF024716)*	Leaves	Salt	Stable	Wang *et al.* 2013
	UBQ7 (DQ116441)				
	CYP1(GQ292530)	Roots			
	α-Tubulin 10				
	UBQ7, GAPDH, EF-1α 8, α-Tubulin 10	Leaves, root	Salt, drought		
	HIS3, UBQ7, UBQ1(EU604080),	Leaves	Drought		
	EF-1α 8 (DQ174257)				
	Actin14 (AY305733)	Salt, drought		Unstable	
	eIF5 (CO492947)				
	CYP1				
	Actin 7	ABA, cold, drought, salt and alkalinity		Stable	Zhu *et al.* 2013; Liang *et al.* 2016
	Actin	Leaf senescence			Lin *et al.* 2015
	Actin1	Hormone, salt, drought, H2O2, wounding, cold, leaf senescence			Evans *et al.* 2016
	UBQ	Wounding, salt, drought			Dongdong *et al.* 2016
	eEF1-α	Cold, drought, salt, ABA			Meng *et al.* 2009
	Biotic stress				
	Actin 4, PP2A1	*Helicoverpa armigera*		Stable	Huang *et al.* 2015
	UBQ	*Aphis gossypii Bemisia tabacci*			Dubey *et al.* 2013
Glycine max	**Abiotic stress**				
	60S (Glyma17g05270)	Root, shoot	Dehydration, salt, ABA, cold	Stable	Le *et al.* 2012
	F-BOX (Glyma12g05510)				
	eEF1- β (Glyma02g44460)				
	ABC(Glyma12g02310)				
	IDE (AW310136)				
	CYP2 (Glyma12g02790), ABC, eEF1-β, 60S (Glyma18g52780)	Root			
	Actin11 (Glyma18g52780),	Shoot			
	F-BOX, IDE				
	Tubulin α (Glyma05g29000)	Root, shoot		Unstable	
	UBQ (Glyma13g17830)				
	CDPK (Glyma10g38460)				
	β-Actin, 18SrRNA, Lectin	Dehydration		Stable	Moreira *et al.* 2011
	β-Tubulin	Cold, ABA, dehydration, salt			Cheng *et al.* 2009
	F-BOX, Actin, α-Tubulin	Dehydration stress			Rodrigues *et al.* 2010
	Biotic stress				
	Actin	*Fusarium oxysporum*		Stable	Lanubile *et al.* 2015
	UBQ3, miR156a (MIMAT0001686), miR156b (MIMAT0001692),				Tremblay *et al.* 2011

Table 1. continued

	miR1520d (MIMAT0007379)	*Phakopsora pachyrhizi*		Rodrigues *et al.* 2010
	miR167c (MIMAT0007355)		Unstable	
	miR171b (MIMAT0007363)			
	Different tissues and Genotypes			
	miR156b, miR1520d	3 genotypes, Roots and leaves from the 'Embrapa 48' genotype	Stable	
	α-Tubulin (CA801144)		Unstable	
	CDPK (AW396185)			
	F-BOX (CD397253)			
	miR396a (MIMAT0001687)			
	eEF1-α (TC203954)	Different tissues, developmental stages, photoperiod treatments, different cultivar	Stable	Jian *et al.* 2008
	CYP2 (TC224926)			
	Actin11 (TC204137)			
	UBQ10 (TC203625)			
	GAPDH (TC224599)			
	β-Tubulin (TC203804)		Unstable	
Saccharum Sp.	**Abiotic stress**			
	eEF1-α	ABA, MeJA, and SA	Stable	Ling *et al.* 2014
	(EF581011.1)			
	GAPDH (CA254672)			
	CUL (C574093.1)			
	TIPS-4(CA228782.1)		Unstable	
	UBQ (CA262530.1)			
	β-Tubulin (CA222437)			
	CAC (CA203604.1)	H_2O_2, NaCl, PEG, $CuCl_2$ and $CdCl_2$	Stable	
	CUL			
	APRT (CA089592.1)			
	TIPS-41		Unstable	
	β-Actin (CA148161)			
	18SrRNA(SCFRRE06)			
	GAPDH, CUL, eEF1-α, β-Tubulin, TIPS-41, UBQ, APRT	ABA, MeJA, SA, H_2O_2, NaCl, PEG, $CuCl_2$ and $CdCl_2$		
	eEF1-α, GAPDH, eIF-4a, TIPS-41, 18SrRNA, β-Actin	Salt, drought	Stable	Guo *et al.* 2014
			Unstable	
	Organs			
	CAC, CUL, UBQ, β-Actin, PRR	Leaf, leaf sheath, stem epidermis, stem pith and bud from different cultivars	Stable	Ling *et al.* 2014
			Unstable	
	25S rRNA(CA171131), GAPDH, β-Actin, β-Tubulin	Iimmature leaf roll, lamina from fully expanded leaf, stem, internodes, roots	Stable	Iskandar *et al.* 2004

Table 1. continued

			Unstable	
Daucus carota	**Abiotic stress**			
	Actin, eIF-4α, GAPDH	Drought stress, salt stress, cold, heat and hormone treatment	Stable	Tian *et al.* 2015
	UBQ, EF-1α, TIP41	Drought stress, salt stress, cold and heat treatment		
	eIF-4α		Unstable	
	SAND, PP2A	Drought stress, salt stress, cold, heat and hormone treatment		
	Actin, β-Tubulin	Dehydration stress	Stable	Perrin *et al.* 2017
	Biotic stress			
	Actin, β-Tubulin, GAPDH	*Alternaria dauci*	Stable	Perrin *et al.* 2017; Boedo *et al.* 2008
Actinidia delicosa	**Biotic stress**			
	GAPDH, PP2A	*Pseudomonas syringae* pv. *actinidiae*	Stable	Petriccione *et al.* 2015
	β-Tubulin		Unstable	
	7s-globulin			
	Abiotic stress			
	Actin	Illumination treatments, hormonal treatments, heat, waterlogging, post-harvest, high CO2, water loss	Stable	Li *et al.* 2013a; Li *et al* . 2013b; Yin *et al.* 2012
Vitis vinifera	**Biotic stress**			
	VATP16 (XM_002269086.1)	Berries	*Plasmopara viticola, Botrytis cinerea*	Stable	Gamm *et al.* 2011
	60S RPL18 (XM_002270599.1)			
	UBE2 (XM_002275879)			
	18S rRNA (GQ849399)			Unstable
	VPS54 (XM_002272141.1)			
	UQCC (XM_002264785.1)			
	VATP16, 60S RPL18, UQCC, 18S rRNA, UBE2,39S RP (XM_002285709.1)	Leaves		Stable
				Unstable
	Abiotic stress			
	Actin	Salt stress and osmotic stress	Stable	Ma *et al.* 2015
	Developmental stages			
	AIG1	Anthesis, fruit-setting, 6–8 mm berries	Stable	González-Agüero *et al.* 2013
	(XM_002281960)			
	TCPB			
	(XM_002285876)	Grape berries at different stages		Wang *et al.* 2012
	Actin1, Actin	Grape berries		Pilati *et al.* 2007

Table 1. continued

Coffea arabica	**Biotic stress**				
	GAPDH	*Hemileia vastatrix*		Stable	Barsalobres-Cavallari *et al.* 2009
	(SGN-U347734)				
	3/14/2003				
	(SGN-U356404)				
	60SRPL7			Unstable	
	(SGN-U351477)				
	ADH (SGN-U350348)				
	UBQ (SGN-U347154)				
	Actin 7				
	Organs				
	GAPDH, 14-3-3, 60S RPL7, ADH, UBQ, Actin 7	Root, stem, leaf, three different stages of flower development, five different kinds of coffee cherries		Stable	
				Unstable	
Solanum tuberosum	**Abiotic stress**				
	CYP (AF126551)	Salt		Stable	Nicot *et al.* 2005
	18SrRNA (X67238)	Salt, cold			
	eEF1-α (AB061263)				
	RPL2 (39816659)	Cold			
	Actin (X55749)				
	β-Tubulin (609267)	Salt, cold		Unstable	
	eEF1-α	Dehydration, high light		Stable	Szalonek *et al.* 2015
	Biotic stress				
	eEF1-α, 18SrRNA,	Late blight		Stable	Nicot *et al.* 2005
	β-Tubulin				
	Actin			Unstable	
	eEF1-α	*Fusarium solani*		Stable	Charfeddine *et al.* 2016
Cucumis sativus	**Abiotic stress**				
	CYP (AY942800)	Root	Different sources of nitrogen (glutamate, glutamine or NH3)	Stable	Warzybok and Migocka 2013
	TIP41(GW881871)				
	Expressed protein	Stem			
	(GW881873)				
	CACS (GW881874)				
	EF-1α (EF446145)	Leaf			
	Actin (AB010922)				
	Expressed protein, TIP41, CYP, YSL8	All organs together			
				Unstable	
	TIP41	Root	Nitrogen deprivation, varying nitrate availability	Stable	
	UBQ (AF104391)				
	Actin, F-box	Stem			
	(GW881870)				
	EF-1α	Leaf			
	TIP41				

Table 1. continued

	CACS, CYP, PDF2	All organs together	Unstable	
	UBQ	Hormones, wounding, cold, salt	Stable	Yang *et al.* 2012
	Actin	Photooxidative stress		Li *et al.* 2013b
	CACS, F-box, TIP41	ABA, salt, drought		Gan *et al.* 2017
	EF-1α	Heat, cold, hormones,dehydration		Wang *et al.* 2015
	Biotic stress			
	UBQ	*Phytophthora melonis, P. capsici*	Stable	Wu *et al.* 2014b
	Actin	*Fusarium oxysporum*		Zhou and Wu 2009
	EF-1α	*Pseudoperonospora cubensis*		Wang *et al.* 2015
Solanum lycopersicum	**Different organs during various developmental stages**			
	CAC (SGN-U314153)	Primary root, mature root, cotyledons, leaf samples, inflorescence, seeds and pericarp at different developmental stages	Stable	Expósito-rodríguez *et al.* 2008
	TIP41(SGN-U321250)			
	Expressed			
	(SGN-U346908)			
	SAND(SGN-U316474)			
	eEF1-α (X53043)		Unstable	
	α-tubulin(AC122540)			
	GAPDH (U97257)			
	Organs			
	DnaJ-like (TC124053)	Leaf, root, fruit and flower	Stable	Coker and Davies. 2003
	TCTP (TC115845)			
	α-tubulin (TC115716)			
	CYP (TC115937)			
	GAPDH (TC115908)			
	Catalase (TC115865)		Unstable	
	Cys protease (TC124125)			
	UDP glucose PTG			
	Chaperonin-60 β			
	Abiotic stress			
	Actin (TC194780)	Nitrogen stress, cold, light	Stable	Løvdal and Lillo 2009
	β-tubulin (DQ205342)			
	RPL2 (X64562)			
	PP2A (AY325817)			
	GAPDH		Unstable	
	PGK (TC203809)			
	eEF1-α (X14449)			
	Biotic stress			
	GAPDH (U93208)	Plant viruses (*CMV-Fny, TYLCV, PVY, CMV-77satRNA, ToMV-SP, TSWV*) and a viroid (PSTVd)	Stable	Mascia *et al.* 2010
	Actin (BT013707)			
	Uridylate kinase			
	UBQ 3 (X58253)			
	18S rRNA (X51576)		Unstable	
	eEF1-α (X53043)			
	Different tissues, developmental stages, temperature stress			
	PTPSec23A, 26S proteasome, AAA-superfamily, ARF, RNase L inhibitor,	Root, shoot, stem, leaves, spike, floral organs and caryopses at	Stable	

Table 1. continued

Triticum aestivum	*Hypothetical,*	different developmental stages, temperature stress A1		Paolacci *et al.* 2009
	UQCR Fe-S			
	Histone, RP,β-Tubulin, Zn-finger, Actin, A20-like		Unstable	
	Abiotic stress			
	Actin	Salt, heat, freezing, drought, ABA	Stable	Goyal *et al.* 2016; Qin *et al.* 2008; Zhu *et al.* 2014; Dong *et al.* 2012
	α-tubulin	Hormones, salt, heat		Han *et al.* 2012
	5SrRNA, APTI	Drought, heat, salt		Pandey *et al.* 2014
	Biotic stress			
	eEF1-α, β-Tubulin	*Blumeria graminis* f. sp. *tritici*	Stable	Tayeh *et al.* 2014
	Actin	*Rhizoctonia cerealis*		Zhu *et al.* 2014

*Expansion of gene names are given in supplementary table 1.

**Gene IDs are not given for genes whose ID is not provided in the respective references.

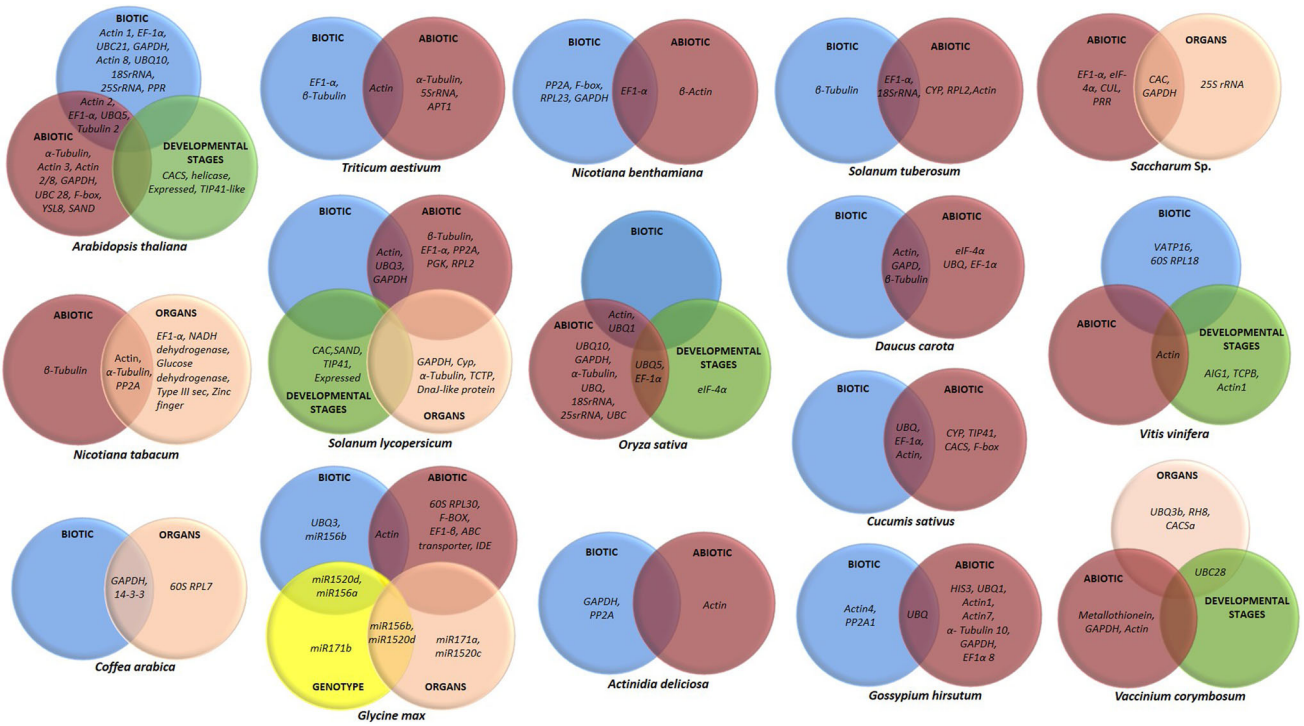

Figure 1. Reference genes that displayed stable expression (for exceptions, see Table 1) in different plants under various conditions i.e., biotic and abiotic stress, organs, developmental stages and genotypes.

becomes a tedious process. For instance, in Nicotiana benthamiana, out of the 16 housekeeping genes analyzed, only 3 genes exhibited uniform expression upon viral infection (Liu et al. 2012). Similarly, out of the 10 genes analyzed in rice, only 2 showed uniformity (Jain et al. 2006) under various abiotic stresses. Also, more the types of treatments to be compared, more the variation and hence, more difficult would it become to identify an appropriate reference gene.

There are reports of about 120 genes analysed for their potential as reference genes under various conditions, across 16 different plants (table 1). Not all expressed stably. Even among the stably expressing genes, there are very few candidates that shows stable expression across various categories of treatments like biotic and abiotic stress, developmental stage, etc. (figure 1).

6. Conclusion

Identifying appropriate reference gene is always the stepping stone for any expression analysis under a new treatment. More the number of treatments, more the variation and hence less the probability for identifying appropriate reference gene. This review serves as a guide for selecting reference genes in plants. We have reviewed all the reference genes that have been used for expression analyses in 16 different plants (Arabidopsis thaliana, Gossypium hirsutum, Nicotiana benthamiana, Nicotiana tabaccum, Glycine max, Oryza sativa, Vaccinium corymbosum, Solanum lycopersicum, Triticum aestivum, Solanum tuberosum, Saccharum sp., Daucus carota, Coffea arabica, Cucumis sativus, Actinidia deliciosa and Vitis vinifera) under any/all of the four different conditions such as biotic stress, abiotic stress, developmental stages and different organs and tissues, reported till date. About 120 genes have been tested across these plants for their stability by various groups (table 1). Even the routinely used reference genes show variable expression due to minute changes in the experimental setup, as reflected in the table 1 (see supplementary table 1 for complete details). Therefore, thorough evaluation of reference gene stability is strongly recommended before performing any expression analysis in order to get rid of false interpretations. For quick reference, we have categorised the stably expressing genes based on the conditions like abiotic and biotic stress, development, etc. in these 16 plants (figure 1). Depending on the type of the experiment, the researcher may include two or three reference genes to determine the relative amount of input cDNA used in the experiment. We believe that this review will certainly ease job of reference gene hunting in any new experimental condition in plant research.

References

Archana K, Rama N, Mamrutha HM and Nataraja KN 2009 Down-regulation of an abiotic stress related Nicotiana benthamiana WRKY transcription factor induces physiological abnormalities. Ind. J. Biotechnol. 8 53–60

Atkinson NJ, Lilley CJ and Urwin PE 2013 Identification of genes involved in the response of Arabidopsis to simultaneous biotic and abiotic stress. Plant Physiol. 162 2028–2041

Barsalobres-Cavallari CF, Severino FE, Maluf MP and Maia IG 2009 Identification of suitable internal control genes for expression studies in Coffea arabica under different experimental conditions. BMC Mol. Biol. 10 1

Belhaj K, Cano LM, Prince DC, Kemen A, Yoshida K, Dagdas YF, Etherington GJ, Schoonbeek H, van Esse HP, Jones JDG, Kamoun S and Schornack S 2017 Arabidopsis late blight: Infection of a nonhost plant by Albugo laibachii enables full colonization by Phytophthora infestans. Cell. Microbiol. 19 e12628

Blancheteau CI, Aquea F, Reyes-Díaz M, Alberdi M and Arce-Johnson P 2011 Identification of Aluminium-regulated genes by cDNA-AFLP analysis of roots in two contrasting genotypes of highbush blueberry (Vaccinium corymbosum L.). Mol. Biotechnol. 49 32–41

Boedo C, Le Clerc V, Briard M, Simoneau P, Chevalier M, Georgeault S and Poupard P 2008 Impact of carrot resistance on development of the Alternaria leaf blight pathogen (Alternaria dauci). Eur. J. Plant Pathol. 121 55–66

Bustin SA 2002 Quantification of mRNA using real-time RT-PCR. Trends and problems. J. Mol. Endocrinol. 29 23–39

Catinot J, Buchala A, Abou-mansour E and Me J 2008 Salicylic acid production in response to biotic and abiotic stress depends on isochorismate in Nicotiana benthamiana. FEBS Lett. 582 473–478

Chapman JR and Waldenström J 2015 With reference to reference genes: a systematic review of endogenous controls in gene expression studies. PLoS One 10 e0141853

Charfeddine M, Bouaziz D, Charfeddine S, Hammami A, Ellouz ON and Bouzid R 2016 Overexpression of dehydration-responsive element binding 1 protein (DREB1) in transgenic Solanum tuberosum enhances tolerance to biotic stress. Plant Biotechnol. Rep. 9 79–88

Chen F, Li Q, Sun L and He Z 2006 The rice 14-3-3 gene family and its involvement in responses to biotic and abiotic stress. DNA Res. 13 53–63

Chen W, Shao J, Ye M, Yu K, Bednarek SY, Duan X, and Guo W 2017 Blueberry VcLON 2, a peroxisomal LON protease, is involved in abiotic stress tolerance. Environ. Exp. Bot. 134 1–11

Cheng L, Huan S, Sheng Y, Hua X, Shu Q, Song S and Jing X 2009 GMCHI, cloned from soybean [Glycine max (L.) Meer.], enhances survival in transgenic Arabidopsis under abiotic stress. Plant Cell Rep. 28 145–153

Coker JS and Davies E 2003 Selection of candidate housekeeping controls in tomato plants using EST data. Plant Cell Physiol. 35 336–339

Czechowski T, Stitt M, Altmann T and Udvardi MK 2005 Genome-wide identification and testing of superior reference genes for transcript normalization. Plant Physiol. 139 5–17

Dean JD, Goodwin PH and Hsiang T 2002 Comparison of relative RT-PCR and northern blot analyses to measure expression of β-1,3-glucanase in Nicotiana benthamiana infected with Colltotrichum destructivum. Plant Mol. Bio. Rep. 20 347–356

Dong W, Ai X, Xu F, Quan T, Liu S and Xia G 2012 Isolation and characterization of a bread wheat salinity responsive ERF transcription factor. Gene 511 38–45

Dongdong L, Ming Z, Lili H, Xiaobo C and Yang G 2016 GhMAPKKK49, a novel cotton (Gossypium hirsutum L.)

MAPKKK gene, is involved in diverse stress responses. Acta Physiol. Plant. 38 1–12

Dubey NK, Goel R, Ranjan A, Idris A, Singh SK, Bag SK, Chandrashekar K, Pandey KD, Singh PK and Sawant SV 2013 Comparative transcriptome analysis of Gossypium hirsutum L. in response to sap sucking insects: aphid and whitefly. BMC Genet. 14 1–20

Dundas J and Ling M 2012 Reference genes for measuring mRNA expression. Theory Biosci. 131 215–223

Egert A, Keller F and Peters S 2013 Abiotic stress-induced accumulation of raffinose in Arabidopsis leaves is mediated by a single raffinose synthase. BMC Plant Biol. 13 218

Evans O, Dou L, Guo Y, Pang C, Wei H, Fan S and Yu S 2016 GhNAC18, a novel cotton (Gossypium hirsutum L.) NAC gene, is involved in leaf senescence and diverse stress responses. Afr. J. Biotechnol. 15 1233–1245

Expósito-rodríguez M, Borges AA, Borges-Pérez A and Pérez JA 2008 Selection of internal control genes for quantitative real-time RT-PCR studies during tomato development process. BMC Plant Biol. 12 1–12

Fauteux F, Chain F, Belzile F, Menzies JG and Belanger RR 2006 The protective role of silicon in the Arabidopsis-powdery mildew pathosystem. Proc. Nat. Acad. Sci. 103 17554–17559

Galletti R, Denoux C, Gambetta S, Dewdney J, Ausubel FM, De Lorenzo G and Ferrari S 2008 The AtrbohD-mediated oxidative burst elicited by oligogalacturonides in Arabidopsis is dispensable for the activation of defense responses effective against Botrytis cinerea. Plant Physiol. 148 1695–1706

Gamm M, Héloir MC, Kelloniemi J, Poinssot B, Wendehenne D and Adrian M 2011 Identification of reference genes suitable for qRT-PCR in grapevine and application for the study of the expression of genes involved in pterostilbene synthesis. Mol. Genet. Genomics 285 273–285

Gan D, Zhan M, Yang F, Zhang Q, Hu K, Xu W, Lu Q, Zhang L and Liang D 2017 Expression analysis of argonaute, Dicer-like, and RNA-dependent RNA polymerase genes in cucumber (Cucumis sativus L.) in response to abiotic stress. J. Genet. 96 235–249

Giri MK, Singh N, Banday ZZ, Singh V, Ram H, Singh D, Chattopadhyay S and Nandi AK 2017 GBF1 differentially regulates CAT2 and PAD4 transcription to promote pathogen defense in Arabidopsis thaliana. The Plant J. 91 802–815

Gong X, Liu M, Zhang L, Ruan Y, Ding R, Ji Y, Zhang N, Zhang S, Farmer J and Wang C 2015 Arabidopsis AtSUC2 and AtSUC4, encoding sucrose transporters, are required for abiotic stress tolerance in an ABA-dependent pathway. Physiol. Plant. 153 119–136

González-Agüero M, García-Rojas M, Di Genova A, Correa J, Maass A, Orellana A and Hinrichsen P 2013 Identification of two putative reference genes from grapevine suitable for gene expression analysis in berry and related tissues derived from RNA-Seq data, BMC Genomics 14 878

Goyal E, Amit SK, Singh RS, Mahato AK and Chand S 2016 Transcriptome profiling of the salt- stress response in Triticum aestivum cv. Kharchia Local. Sci. Rep. 13 27752

Guénin S, Mauriat M, Pelloux J, Van Wuytswinkel O, Bellini C and Gutierrez L 2009 Normalization of qRT-PCR data: the necessity of adopting a systematic, experimental conditions-specific, validation of references. J. Exp. Bot. 60 487–493

Guo J, Ling H, Wu Q, Xu L and Que Y 2014 The choice of reference genes for assessing gene expression in sugarcane under salinity and drought stresses. Sci. Rep. 4 7042

Guo Y, Li R, Asgher Z, Shamsi IH, Yu S, Zhao J and Ren X 2016 Genome-wide selection and testing of superior reference genes for quantitative gene expression normalization in tobacco. J. Biotechnol. Microbiol. 5 68–75

Gutierrez L, Mauriat M, Guénin S, Pelloux J, Lefebvre JF, Louvet R, Rusterucci C, Moritz T, Guerineau F, Bellini C and Van Wuytswinkel O 2008 The lack of a systematic validation of reference genes: a serious pitfall undervalued in reverse transcription-polymerase chain reaction (RT-PCR) analysis in plants. Plant Biotechnol. J. 6 609–618

Han Y, Li A, Li F, Zhao M and Wang W 2012 Characterization of a wheat (Triticum aestivum L.) expansin gene, TaEXPB23, involved in the abiotic stress response and phytohormone regulation. Plant Physiol. Biochem. 54 49–58

Hashimoto M, Kisseleva L, Sawa S, Furukawa T, Komatsu S and Koshiba T 2004 A Novel rice PR10 protein, RSOsPR10, specifically induced in roots by biotic and abiotic stresses, possibly via the jasmonic acid signaling pathway. Plant Cell Physiol. 45 550–559

Huang X, Chen J, Xiao H, Xiao Y, Wu J and Wu J 2015 Dynamic transcriptome analysis and volatile profiling of Gossypium hirsutum in response to the cotton bollworm Helicoverpa armigera. Sci. Rep. 1–14

Huggett J, Dheda K, Bustin S and Zumla A 2005 Real-time RT-PCR normalisation; strategies and considerations. Genes Immun. 6 279–284

Iskandar HM, Simpson RS, Casu RE, Bonnett GD, Maclean DJ and Manners JM 2004 Comparison of reference genes for quantitative real-time polymerase chain reaction analysis of gene expression in sugarcane. Plant Mol. Biol. Rep. 22 325–337

Jain M, Nijhawan A, Tyagi AK and Khurana JP 2006 Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. Biochem. Biophys. Res. Commun. 345 646–651

Jian B, Liu B, Bi Y, Hou W, Wu C and Han T 2008 Validation of internal control for gene expression study in soybean by quantitative real-time PCR. BMC Mol. Biol. 14 1–14

Kim S, Veena G and Gelvin SB 2007 Genome-wide analysis of Agrobacterium T-DNA integration sites in the Arabidopsis genome generated under non-selective conditions. Plant J. 51 779–791

Kozera B and Rapacz M 2013 Reference genes in real-time PCR. J. Appl. Genet. 54 391–406

Lacroix B and Citovsky V 2014 A mutation in negative regulator of basal resistance WRKY17 of Arabidopsis increases susceptibility to Agrobacterium-mediated transient genetic transformation. F1000Research 1–8

Lanubile A, Muppirala UK, Severin AJ, Marocco A and Munkvold GP 2015 Transcriptome profiling of soybean (Glycine max) roots challenged with pathogenic and non-pathogenic isolates of Fusarium oxysporum. BMC Genomics 16 1–14

Le DT, Aldrich DL, Valliyodan B, Watanabe Y and Van Ha C 2012 Evaluation of candidate reference genes for normalization of quantitative RT-PCR in Soybean tissues under various abiotic stress conditions. PLoS ONE 7 e46487

Leng L, Liang Q, Jiang J, Zhang C, Hao Y, Wang X and Su W 2017 A subclass of HSP70s regulate development and abiotic stress responses in Arabidopsis thaliana. J. Plant Res. 130 349–363

Li J, Liang D, Li M and Ma F 2013a Light and abiotic stresses regulate the expression of GDP-l-galactose phosphorylase and levels of ascorbic acid in two kiwifruit genotypes via light-responsive and stress-inducible cis-elements in their promoters. Planta 238 535–547

Li P, Chen L, Zhou Y, Xia X, Shi K, Chen Z and Yu J 2013b Brassinosteroids-induced systemic stress tolerance was associated with increased transcripts of several defence-related genes in the phloem in Cucumis sativus. PLoS ONE 8 1–8

Li L, Zhang H, Liu Z, Cui X, Zhang T, Li Y and Zhang L 2016 Comparative transcriptome sequencing and de novo analysis of Vaccinium corymbosum during fruit and color development. BMC Plant Biol. 223 1–9

Liang C, Meng Z, Meng Z, Malik W, Yan R, Lwin KM, Lin F, Wang Y, Sun G, Zhou T, Zhu T, Li J, Jin S, Guo S and Zhang R 2016 GhABF2, a bZIP transcription factor, confers drought and salinity tolerance in cotton (Gossypium hirsutum L.). Sci. Rep. 6 35040

Lin M, Pang C, Fan S, Song M, Wei H and Yu S 2015 Global analysis of the Gossypium hirsutum L. transcriptome during leaf senescence by RNA-Seq. BMC Plant Biol. 15 1–18

Ling H, Wu Q, Guo J, Xu L and Que Y 2014 Comprehensive selection of reference genes for gene expression normalization in sugarcane by real time quantitative RT-PCR. PLoS ONE 9 e97469

Liu D, Shi L, Han C, Yu J, Li D and Zhang Y 2012 Validation of reference genes for gene expression studies in virus-infected Nicotiana benthamiana using quantitative real time PCR. PLoS ONE 7 e46451

Liu Y, Ji X, Nei X, Qu M, Zheng L, Tan Z, Zhao H, Huo L, Liu S, Zhang B and Wang Y 2015 Arabidopsis AtbHLH112 regulates the expression of genes involved in abiotic stress tolerance by binding to their E-box and GCG-box motifs. New Phytol. 207 692–709

Løvdal T, and Lillo C 2009 Reference gene selection for quantitative real-time PCR normalization in tomato subjected to nitrogen, cold, and light stress. Anal. Biochem. 387 238–242

Ma Y, Wang J, Zhong Y, Geng F, Cramer GR and Cheng ZM 2015 Subfunctionalization of cation/proton antiporter 1 genes in grapevine in response to salt stress in different organs. Hortic. Res. 2 15031

Mascia T, Santovito E, Gallitelli D and Cillo F 2010 Evaluation of reference genes for quantitative reverse-transcription polymerase chain reaction normalization in infected tomato plants. Mol. Plant Pathol. 11 805–816

Meng C, Cai C, Zhang T and Guo W 2009 Characterization of six novel NAC genes and their responses to abiotic stresses in Gossypium hirsutum L. Plant. Sci. 176 352–359

Moreira RS, Gertrudes E, Lemos DM and Abdelnoor RV 2011 Identification of reference genes for expression analysis by real-time quantitative PCR in drought-stressed soybean. Presc. Agropec. Bras. 46 58–65

Msanne J, Lin J and Stone JM 2011 Characterization of abiotic stress-responsive Arabidopsis thaliana RD29A and RD29B genes and evaluation of transgenes. Planta 234 97–107

Naik D, Dhanaraj AL, Arora R, and Rowland LJ 2007 Identification of genes associated with cold acclimation in blueberry (Vaccinium corymbosum L.) using a subtractive hybridization approach. Plant Sci. 173 213–222

Nicot N, Hoffmann L and Lippmann CDRP 2005 Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. J. Exp. Bot. 56 2907–2914

Pandey R, Joshi G, Bhardwaj AR, Agarwal M and Katiyar-Agarwal S 2014 A Comprehensive genome-wide study on tissue- specific and abiotic stress-specific miRNAs in Triticum aestivum. PLoS ONE 9 e95800

Paolacci AR, Tanzarella OA, Porceddu E and Ciaffi M 2009 RT-PCR normalization in wheat. BMC Mol. Biol. 27 1–27

Pecinka A, Rosa M, Schikora A, Berlinger M, Hirt H, Luschnig C and Scheid OM 2009 Transgenerational stress memory is not a general response in Arabidopsis. PLoS ONE 4 e5202

Perrin F, Laurent CD, Gibon Y, Citerne S, Huet S, Suel A, Clerc VL, Briard M, Hamama L, Peltier D, Gagné S and Geoffriau E 2017 Combined Alternaria dauci infection and water stresses impact carotenoid content of carrot leaves and roots. Environ. Exp. Bot. 143 125–134

Petriccione M, Mastrobuoni F, Zampella L and Scortichini M 2015 Reference gene selection for normalization of RT-qPCR gene expression data from Actinidia deliciosa leaves infected with Pseudomonas syringae pv. Sci. Rep. 5 16961

Pfaffl MW 2004 Quantification strategies in real-time PCR; in A-Z of Quantitative PCR (eds) SA Bustin (International University Line: La Jolla, CA, USA) pp 87–112

Pilati S, Perazzolli M, Malossini A, Cestaro A, Demattè L, Fontana P, Dal Ri A, Viola R, Velasco R and Moser C 2007 Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at vèraison. BMC Genomics 8 428

Qin D, Wu H, Peng H, Yao Y, Ni Z, Li Z, Zhou C and Sun Q 2008 Heat stress-responsive transcriptome analysis in heat susceptible and tolerant wheat (Triticum aestivum L.) by using wheat genome array. BMC Genomics 19 1–19

Remans T, Smeets K, Opdenakker K, Mathijsen D, Vangronsveld J and Cuypers A 2008 Normalisation of real-time RT-PCR gene expression measurements in Arabidopsis thaliana exposed to increased metal concentrations. Planta 227 1343–1349

Rocha AJ, Monteiro-Júnior JE, Freire JEC, Sousa AJS and Fonteles CSR 2015 Real Time PCR: the use of reference genes and essential rules required to obtain normalisation data reliable to quantitative gene expression. J. Mol. Bio. Res. 5 45–55

Rodrigues F, Marcelino-guimaraes FC, Lima A, Vilela R and Margis R 2010 The use of microRNAs as reference genes for quantitative polymerase chain reaction in soybean. Anal. Biochem. 406 185–192

Schmidt GW and Delaney SK 2010 Stable internal reference genes for normalization of real-time RT-PCR in tobacco (Nicotiana tabacum) during development and abiotic stress. Mol. Genet. Genomics 283 233–241

Shah JM, Raghupathy V and Veluthambi K 2009 Enhanced sheath blight resistance in transgenic rice expressing an endochitinase gene from Trichoderma virens. Biotechnol. Lett. 2 239–244

Szalonek M, Sierpien B, Rymaszewski W and Gieczewska K 2015 Potato Annexin STANN1 promotes drought tolerance and

mitigates light stress in transgenic Solanum tuberosum L. plants. PLoS ONE 10 e0132683

Tayeh C, Randoux B, Vincent D, Bourdon N and Reignault P 2014 Exogenous trehalose induces defenses in wheat before and during a biotic stress caused by powdery mildew. Phytopathology 104 293–305

Terry BC, Liu X, Murphy AM and Pajerowska-Mukhtar KM 2015 Arabidopsis thaliana GCN2 is involved in responses to osmotic and heat stresses. Int. J. Plant Res. 5 87–95

Tian C, Jiang Q, Wang F, Wang GL, Xu ZS and Xiong AS 2015 Selection of suitable reference genes for qPCR normalization under abiotic stresses and hormone stimuli in carrot leaves. PLoS ONE 10 e0117569

Tremblay A, Hosseini P, Alkharouf NW, Li S and Matthews BF 2011 Gene expression in leaves of susceptible Glycine max during infection with Phakopsora pachyrhizi using next generation sequencing. Sequencing 827250 1–4

Trivedi DK, Ansari MW and Tuteja N 2013 Multiple abiotic stress responsive rice cyclophilin (OsCYP-25) mediates a wide range of cellular responses. Commun. Integr. Biol. 6 1–8

Ueno Y, Yoshida R, Kishi-kaboshi M and Matsushita A 2015 Abiotic stresses antagonize the rice defence pathway through the tyrosine-dephosphorylation of OsMPK6. PLoS Pathog. 11 e1005231

Vaghchhipawala ZE, Vasudevan B, Lee S, Morsy MR and Mysore KS 2012 Agrobacterium may delay plant nonhomologous end-joining DNA repair via XRCC4 to favor T-DNA integration. The Plant Cell 24 4110–4123

Vashisth T and Klima L 2011 An efficient RNA isolation procedure and identification of reference genes for normalization of gene expression in blueberry. Plant Cell Rep. 30 2167–2176

Wang J, Pan C, Wang Y, Ye L, Wu J, Chen L, Zou T and Lu G 2015 Genome-wide identification of MAPK, MAPKK, and MAPKKK gene families and transcriptional profiling analysis during development and stress response in cucumber. BMC Genet. 16 386

Wang M, Wang Q and Zhang B 2013 Evaluation and selection of reliable reference genes for gene expression under abiotic stress in cotton (Gossypium hirsutum L.). Gene 530 44–50

Wang W, Wang HL, Wan SB, Zhang JH, Zhang P, Zhan JC and Huang WD 2012 Chalcone isomerase in grape vine: gene expression and localization in the developing fruit. Biol. Plant. 56 545–550

Warzybok A and Migocka M 2013 Reliable reference genes for normalization of gene expression in cucumber grown under different nitrogen nutrition. PLoS ONE 8 e72887

Woo-Lee C, Efetova M, Engelmann JC, Cramell R, Wasternack C, Ludwig-Muller J, Hedrich R and Deeken R 2009 Agrobacterium tumefaciens promotes tumor induction by modulating pathogen defense in Arabidopsis thaliana. The Plant Cell 21 2948–2962

Wu H, Liu K, Wang Y, Wu J, Chiu W and Chen C 2014a AGROBEST: an efficient Agrobacterium-mediated transient expression method for versatile gene function analyses in Arabidopsis seedlings. Plant Methods 10 1–16

Wu T, Wang R, Xu X, He X, Sun B and Zhong Y 2014b Cucumis sativus L-type lectin receptor kinase (CsLecRK) gene family response to Phytophthora melonis, Phytophthora capsici and water immersion in disease resistant and susceptible cucumber cultivars. Gene 549 214–222

Xin X, Nomura K, Underwood W and He SY 2013 Induction and suppression of PEN3 focal accumulation during Pseudomonas syringae pv. tomato DC3000 infection of Arabidopsis. Mol. Plant-Microbe Interact. 26 861–867

Yan A, Wu M, Yan L, Hu R, Ali I and Gan Y 2014 AtEXP2 is involved in seed germination and abiotic stress response in Arabidopsis. PLoS ONE 9 e85208

Yang X, Jiang W and Yu H 2012 The expression profiling of the lipoxygenase (LOX) family genes during fruit development, abiotic stress and hormonal treatments in cucumber (Cucumis sativus L.). Int. J. Mol. Sci. 13 2481–2500

Yin X, Allan AC, Xu Q, Burdon J, Dejnoprat S, Chen K and Ferguson IB 2012 Postharvest biology and technology differential expression of kiwifruit ERF genes in response to postharvest abiotic stress. Postharvest Biol. Technol. 66 1–7

Yoshimoto N, Takahashi H, Smith FW, Yamaya T and Saito K 2002 Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in Arabidopsis roots. Plant J. 29 465–473

Zhang C, Liu H, Jia C and Liu Y 2016 Cloning, characterization and functional analysis of a flavonol synthase from Vaccinium corymbosum. Trees 30 1595–1605

Zhou X and Wu F 2009 Physiological and molecular plant pathology differentially expressed transcripts from cucumber (Cucumis sativus L.) root upon inoculation with Fusarium oxysporum f. sp. cucumerinum Owen. Physiol. Mol. Plant Pathol. 74 142–150

Zhu X, Qi L, Liu X, Cai S, Xu H, Huang R, Li J, Wei X and Zhang Z 2014 The wheat ethylene response factor transcription factor pathogen-induced ERF1 mediates host responses to both the necrotrophic pathogen. Plant Physiol. 164 1499–1514

Zhu Y, Shi D, Ruan M, Zhang L, Meng Z, Liu J and Yang W 2013 Transcriptome analysis reveals crosstalk of responsive genes to multiple abiotic stresses in cotton (Gossypium hirsutum L.). PLoS ONE 8 e80218

Corresponding editor: Ashis Kumar Nandi