

**Table 1a:** Important studies concerned on validation of references in different plant species

Plant	Reference gene	Stable reference gene	Reference
<i>O. sativa</i>	<i>GAPDH</i> , <i>ACT</i> , <i>β-TUB</i> and <i>18S rRNA</i>	<i>18S rRNA</i>	Kim et al., 2003
<i>Saccharum</i> sp.	<i>β-ACT</i> , <i>β-TUB</i> , <i>GAPDH</i> and <i>25S rRNA</i> Target gene: <i>Pst2a</i> (sugar transporter gene)	<i>GAPDH</i> and <i>25S rRNA</i>	Iskandar et al. 2004
Poplar ( <i>Populus trichocarpa</i> × <i>P. deltoides</i> )	<i>ACT11</i> , <i>ACT2</i> , <i>CYP</i> , <i>TUA</i> , <i>TUB</i> , <i>UBQ</i> , <i>UBQ-L</i> , <i>EIF4B-L</i> , <i>EF1β</i> and <i>18S rRNA</i>	<i>TUA</i> and <i>UBQ</i>	Brunner et al., 2004
Potato	Reference gene- <i>b-TUB</i> , <i>ef1a</i> , <i>L2</i> (Cytoplasmic ribosomal protein), <i>18S rRNA</i> , <i>aprt</i> (Adenine phosphoribosyl transferase), <i>ACT</i> and <i>CYP</i> Target gene ( <i>Hsp20.2</i> )	Late blight ( <i>ef1a</i> and <i>18S rRNA</i> )/Cold stress ( <i>ef1a</i> and <i>L2</i> )/Salt stress ( <i>ef1a</i> and <i>CYP</i> )/Internal control for <i>Hsp20.2</i> ( <i>18S rRNA</i> and <i>ef1a</i> ) Least stable: Actin and tubulin (Abiotic stress)/Actin and <i>aprt</i> (Biotic stress)	Nicot et al. 2005
<i>O. sativa</i>	<i>18S rRNA</i> , <i>25S rRNA</i> , <i>UBC</i> , <i>UBQ5</i> , <i>UBQ10</i> , <i>ACT11</i> , <i>GAPDH</i> , <i>eEF-1a</i> , <i>eIF-4a</i> , and <i>β-TUB</i>	Most stable: <i>UBQ5</i> and <i>eEF-1a</i> / Least stable: <i>UBQ10</i> , <i>ACT11</i> and <i>β-TUB</i>	Jain et al., 2006
<i>V. vinifera</i>	<i>ACT</i> , <i>AP47</i> , <i>CYP</i> , <i>EF-1a</i> , <i>GAPDH</i> , <i>MDH</i> , <i>PP2A</i> , <i>SAND</i> , <i>TIP41</i> , <i>α-TUB</i> , <i>β-TUB</i> , <i>UBC</i> , <i>UBQ-L40</i> and <i>UBQ10</i>	<i>GAPDH</i> , <i>EF-1a</i> and <i>SAND</i>	Reid et al., 2006
<i>O. sativa</i>	2500 transcription factors and seven common references ( <i>ACT</i> , <i>ACT1</i> , <i>β-TUB</i> , <i>CYP</i> , <i>eF1a</i> , <i>TIP41</i> -like, Expressed protein)	<i>ACT1</i> , <i>EF-1a</i> and <i>EP</i>	Caldana et al. 2007
<i>L. temulentum</i>	<i>ACT11</i> , <i>eEF-1a</i> , <i>eIF-4a</i> , <i>GAPDH</i> , <i>b-TUB</i> , <i>UBC</i> , <i>UBQ5</i> , <i>25S rRNA</i> and <i>CAP</i> (cap binding protein)	<i>eEF-1a</i> and <i>UBQ5</i>	Dombrowski and Martin, 2009
<i>O. ramosa</i>	Reference gene ( <i>18S rRNA</i> , <i>act1</i> , <i>tub1</i> , and <i>ubq1</i> ) and target gene ( <i>MYB1</i> )	<i>act1</i> and <i>ubq1</i>	González-Verdejo et al. 2008
<i>G. max</i>	Reference gene- <i>ACT11</i> , <i>ACT2/7</i> , <i>TUA</i> , <i>TUB</i> , <i>UBC2</i> , <i>CYP2</i> , <i>G6PD</i> , <i>EF-1a</i> , <i>EF-1β</i> and <i>UBQ10</i> and Target gene ( <i>GmBFT</i> ( <i>Glycine max</i> brother of FT and TFL1))	Most stable ( <i>EF-1β</i> and <i>CYP2</i> ) / Stable across cultivar ( <i>UBC2</i> ) Least stable ( <i>UBQ10</i> and <i>G6PD</i> )	Jian et al. 2008
<i>S. lycopersicon</i>	Reference gene : <i>GAPDH</i> , <i>eF1a</i> , <i>TBP</i> , <i>TUA</i> , <i>RPL8</i> , <i>APT</i> , <i>DNAJ</i> and Target gene: <i>TIP41</i> (SGN-U321250), <i>SAND</i> (SGN-U316474), <i>CAC</i> (SGN-U314153) and Expressed (SGN-U346908)	Most stable: <i>CAC</i> and <i>TIP41</i> / Moderate stable: "Expressed", <i>TBP</i> and <i>SAND</i> / Least stable: <i>GAPDH</i> , <i>eF1a</i> and <i>TUA</i>	Expósito-Rodríguez et al. 2008
<i>B. distachyon</i>	<i>ACT7</i> , <i>RCA</i> ( <i>rubisco</i> activase), <i>eF1a</i> , <i>GAPDH</i> , <i>TUA6</i> , <i>UBC18</i> , <i>Ubi4</i> , <i>Ubi10</i> and <i>SamDC</i> (S-adenosyl methionine decarboxylase)	Overall ( <i>UBC18</i> ) / Growth hormone ( <i>Ubi4</i> and <i>Ubi10</i> ) / Abiotic stresses ( <i>SamDC</i> )	Hong et al. 2008
Sunflower	<i>18S rRNA</i> , <i>26S rRNA</i> , <i>UBQ13</i> , <i>TUB2</i> , <i>TUB1</i> , <i>PEP</i> and <i>EF-1</i>	<i>TUB1</i>	Fernandez et al. 2008
<i>A. thaliana</i>	Reference gene: <i>ACT2</i> , <i>ACT</i> , <i>ACT8</i> , <i>APT1</i> , <i>eF1a</i> , <i>eIF4A</i> , <i>TUB2</i> , <i>TUB6</i> , <i>TUB9</i> , <i>UBQ4</i> , <i>UBQ5</i> , <i>UBQ10</i> , <i>UBQ11</i> and <i>NDUFA8</i> Target gene [ <i>eIF4A</i> , <i>At5g02840</i> (MYB factor) and <i>LEAFY COTYLEDON1-Like</i> ( <i>LIL</i> )] Gene tested across different plant species <i>At4g34270</i> and <i>At4g33380</i>	Most stable ( <i>APT1</i> , <i>UBQ5</i> , <i>eF1a</i> ) Gene stable across different plant species ( <i>At4g34270</i> and <i>18S rRNA</i> )	Gutierrez et al. 2008

	( <i>Arabidopsis</i> ), UBQ (Poplar) and 18S rRNA (Aspen)		
<i>A. thaliana</i>	Ten most stably expressed genes of <i>Arabidopsis thaliana</i> identified by Czechowski et al. (2005) Three traditional HKGs ( <i>EF-1a</i> , <i>ACT2</i> and <i>UBQ10</i> )	Most stable : <i>AT5G15710</i> (F-box protein), <i>AT2G28390</i> (SAND family protein), and <i>AT5G08290</i> (mitosis protein YLS8)	Remans et al. 2008
<i>C. arabica</i>	5 traditional HKGs ( <i>actin</i> , <i>adh</i> , <i>14-3-3</i> , <i>gapdh</i> , <i>poly</i> ) Novel genes [ <i>Cysteine proteinase (cys)</i> , <i>Caffeine synthase (ccs)</i> and the 60S ribosomal protein L7 ( <i>rpl7</i> )]	<i>gapdh</i> , <i>14-3-3</i> and <i>rpl7</i>	Barsalobres-Cavallari et al. 2009
<i>B. brizantha</i>	<i>ef1a</i> , <i>eIF-4a</i> , <i>GAPDH</i> , <i>GDP</i> , <i>TUB</i> , <i>UBI</i> , <i>SUCOA</i> (succinyl-CoA ligase) and <i>UBCE</i> (ubiquitin conjugating enzyme)	Stable reference ( <i>UBCE</i> and <i>ef1a</i> ) Least stable ( <i>TUB</i> and <i>SUCOA</i> )	Silveira et al. 2009
<i>C. arabica</i>	<i>GAPDH</i> , <i>UBQ10</i> , <i>UB19</i> , <i>PP2A</i> , <i>AP47</i> , <i>S24</i> (40S ribosomal subunit) and <i>psaB</i> (D1 subunit of photosystem I and II reaction centre)	<i>UB19</i> , <i>PP2A</i> , <i>AP47</i> , <i>S24</i> (observed for different data set)	Cruz et al. 2009
<i>O. sativa</i>	Reference gene ( <i>18SrRNA</i> , <i>25S rRNA</i> , <i>eF1a</i> , <i>eIF-4a</i> , <i>UBC</i> , <i>UBQ5</i> , <i>ACT1</i> , <i>GADPH</i> and <i>TBL</i> ) and Target genes (Seed development specific): <i>GBSSI</i> , <i>SSSI</i> , <i>SBE IIb</i> and <i>ISA1</i>	<i>eIF-4a</i> and <i>ACT1</i>	Li et al. 2010
<i>G. max</i>	Traditional references ( <i>ACT2</i> , <i>ACT11</i> , <i>TUB4</i> , <i>TUA5</i> , <i>CYP</i> , <i>UBQ10</i> , <i>EF1b</i> ) / New references ( <i>SKIP16</i> , <i>MTP</i> , <i>PEPKR1</i> , <i>HDC</i> , <i>TIP41</i> , <i>UKN1</i> , <i>UKN2</i> ) / Target reference <i>GmFTL3</i> (ortholog of <i>Arabidopsis FT</i> (flowering locus T))	Overall ( <i>SKIP16</i> , <i>UKN1</i> and <i>UKN2</i> ), developmental stages ( <i>SKIP16</i> , <i>UKN1</i> and <i>MTP</i> ), photoperiods ( <i>ACT11</i> , <i>TUA5</i> and <i>TIP41</i> ), light quality ( <i>TIP41</i> , <i>UKN1</i> and <i>UKN2</i> ), cultivars for photoperiods ( <i>ACT11</i> , <i>UKN2</i> and <i>TUB4</i> )	Hu et al. 2009
<i>L. perenne</i>	<i>ACT11</i> , <i>CAP</i> , <i>eEF-1a</i> , <i>eIF-4a</i> , <i>GAPDH</i> , $\beta$ - <i>TUB</i> , <i>UBC</i> , <i>UBQ5</i> and <i>25SrRNA</i>	Overall: <i>eEF-1a</i> and <i>eIF-4a</i> / Root crown tissues: <i>eIF-4a</i> and <i>25S rRNA</i> (most stable) / <i>CAP</i> (Least stable) / For leaf samples: <i>eEF-1a</i> and <i>UBQ5</i> (most stable) / $\beta$ - <i>TUB</i> (Least stable)	Martin et al. 2008
<i>A. thaliana</i> <i>Picea abies</i>	For <i>Arabidopsis</i> : <i>ACT</i> , adenine phosphoribosyltransferase 1, RNA polymerase II large subunit and ubiquitin For <i>Picea abies</i> : <i>UBQ</i> , 18S rRNA and <i>TUB</i>	The study was conducted to evaluate the efficiency of purified cDNA	Phillips et al. 2009
<i>S. miltiorrhiza</i>	Reference gene ( <i>18SrRNA</i> , <i>eF1a</i> , <i>ACT</i> , $\alpha$ - <i>TUB</i> , <i>UBQ</i> ) and Target gene ( <i>SmDXR</i> )	Stable references ( <i>UBQ</i> and <i>ACT</i> ) Least stable ( <i>18SrRN</i> and <i>eF1a</i> )	Yang et al. 2010
<i>P. persica</i>	<i>18S rRNA</i> , <i>ACT</i> , <i>CYP2</i> , <i>TEF2</i> , <i>GAPDH</i> , <i>PLA2</i> , <i>RP II</i> , <i>RPL13</i> , <i>TUA</i> , <i>TUB</i> and <i>UBQ10</i>	Most stable ( <i>TEF2</i> , <i>UBQ10</i> and <i>RP II</i> ) and least stable ( <i>18S rRNA</i> , <i>RPL13</i> , <i>PLA2</i> <i>GAPDH</i> and <i>ACT</i> )	Tong et al. 2009
<i>S. lycopersicum</i>	Traditional reference gene ( <i>ACT</i> , <i>TUB</i> , <i>ef1a</i> , <i>GAPDH</i> , <i>PGK</i> and <i>UBI</i> ) Novel gene ( <i>RPL2</i> and <i>PP2Acs</i> )	Light stress ( <i>GAPDH</i> and <i>PGK</i> ) Nitrogen and cold stress ( <i>ef1a</i> )	Løvdal and Lillo, 2009
<i>O. sativa</i>	Traditional reference gene Eight paralogous genes of actin ( <i>ACT</i> ), <i>eEF-1a</i> and $\beta$ - <i>TUB</i> / Target gene <i>GS3</i> (a QTL for grain length and weight)	<i>ACT</i> and <i>eEF-1a</i>	Zhang et al. 2009
Swingle citrumelo citrus rootstock	Reference gene <i>ACT</i> , <i>TUB</i> , <i>ef1a</i> , <i>GAPDH</i> , ADP ribosylation factor ( <i>ADP</i> ), <i>CYP</i> , Cathepsin ( <i>CtP</i> ) / Target gene $\Delta^1$ -pyrroline-5-carboxylate synthetase ( <i>P5CS</i> )	<i>ef1a</i> and <i>ADP</i>	Carvalho et al. 2010
<i>C. arietinum</i>	HSK references ( <i>ACT1</i> , <i>EF-1a</i> , <i>GAPDH</i> , <i>IF4a</i> , <i>TUB6</i> , <i>UBC</i> , <i>UBQ5</i> , <i>UBQ10</i> , <i>18SrRNA</i> , <i>25SrRNA</i> )	Most stable ( <i>EF1a</i> and <i>HSP90</i> ) Stable for stresses ( <i>IF4a</i> and <i>GAPDH</i> )	Garg et al. 2010

	Novel references ( <i>GRX and HSP90</i> )		
<i>G. hirsutum</i>	<i>ACT4, ef1α, FBX6, PP2A1, MZA, PTB, GAPC2, βTUB3 and UBQ14</i> Two cotton MADS-box genes for validation ( <i>MADS3 and SEP-like1</i> )	Most stable ( <i>PP2A1 and UBQ14</i> ) and stable during flower development ( <i>ACT4 and UBQ14</i> ), in floral verticils ( <i>ACT4 and FBX6</i> ), in fruit development ( <i>MZ and, PTB</i> )	Artico et al. 2010
Cucumber ( <i>C. hystrix/C. sativus</i> )	<i>ACT, ACT1, ACT2, ACT3, ef1α, 18SrRNA, CYP, TUA, UBI1 and UBI-ep</i>	<i>TUA, ef1α, UBI-ep</i>	Wan et al. 2010
<i>P. hybrid</i> (Mitchell line and V30 line)	<i>ACT11, CYP2, EF1α, UBQ, GAPDH</i> , GTPbinding protein RAN1 ( <i>RAN1</i> ), SAND protein ( <i>SAND</i> ), Ribosomal protein S13 ( <i>RPS13</i> ) and ( <i>TUB6</i> )	Mitchell line ( <i>EF1α and SAND</i> ) / V30 line ( <i>CYP and RAN1</i> ) Least adequate reference gene ( <i>GAPDH</i> )	Mallona et al. 2010
<i>C. intybus</i>	Reference genes ( <i>NADHD, ACT, TUB, GAPDH, H3(Histone H3), ef1α, 18SrRNA</i> ) and Target genes ( <i>FEHIIa and FEHIIb</i> )	<i>ACT, ef1α, 18S rRNA</i>	Maroufi et al. 2010
Wheat, Barley, Oat	Reference gene ( <i>GAPDH, TUA, TUB, EF1α, eIF4A, 18S rRNA, 28S rRNA</i> ) and Target gene ( <i>BYDV cp</i> )	Barley: <i>GAPDH, 18S rRNA</i> and <i>TUB/Oat: GAPDH, 18S rRNA, TUB</i> and <i>TUA/Wheat: GAPDH, 18S rRNA, TUB</i> and <i>eIF4A</i>	Jarošová and Kundu, 2010
<i>Eucalyptus</i>	Reference gene: <i>GAPDH, TUB, eEF2α, ACT, CYP, UBQ, PUBQ, eIF4β, 18S rRNA, 28S rRNA, APRT, Hsp20</i> and <i>EgIDH</i> /Target gene: Chitinase gene ( <i>ASM</i> and <i>P. psidii</i> treatments)	Tissue not received any stimuli: <i>eEF2/EgIDH</i> <i>P. psidii</i> -inoculated and uninoculated: <i>eEF2 and UBQ</i> ASM-treated and untreated: <i>CYP and eIF4B</i>	Boava et al. 2010
<i>L. perenne</i>	Reference gene: <i>eEF1A</i> (m), <i>eEF1A</i> (h), <i>eEF1A</i> (s), <i>TBP-1, eIF4A, YT521-B</i> ( <i>YT521-B</i> -like family protein), <i>H3</i> (Histone 3) and <i>E2</i> (Ubiquitin-conjugating enzyme)/Target gene: chloroplast translational elongation factor Tu ( <i>EF-Tu</i> )	<i>eEF1A</i> (s) and <i>YT521-B</i> <i>H3</i> was removed from study because of low expression	Lee et al. 2010
<i>Nymphaea spp.</i>	<i>ACT11, GAPDH, ef1α, UBC16, IF1, UBQ11, Ribosomal protein S1 (RPS1)</i> and <i>Clathrin adaptor complexes medium subunit (AP47)</i>	geNorm: <i>AP47 &amp; ACT11</i> (All samples) NormFinder: <i>AP47 &amp; ef1α</i>	Luo et al. 2010
<i>B. chinense</i>	Reference gene - <i>ACT, α-TUB, β-TUB, CYP, RPL2, EF1α, 18S rRNA</i> , and Target gene - <i>HMGR, IPPI, FPS</i> and <i>β-AS</i>	Most stable - <i>β-TUB</i> Least stable - <i>18S rRNA, CYP, ef1α</i> ,	Dong et al. 2010
<i>L. usitatissimum</i>	Reference gene - <i>ACT, CYP, EF1α, GAPDH, UBI, UBI2, TUA, EF2, ETIF1, ETIF3E, ETIF3H, ETIF4F</i> and <i>ETIF5A</i> and Target gene - <i>LuMYB1</i>	Most stable – NormFinder ( <i>GAPDH</i> ) geNorm ( <i>EF1A, ETIF5A, Ubiquitin</i> )	Huis et al. 2010
<i>Eucalyptus globules</i> ( <i>In vitro</i> )	Reference gene - <i>18S rRNA, ACT2, EF2, EUC12, H2B, IDH, SAND, TIP41, TUA, UBI</i> and <i>AT4G33380</i> and Target gene - <i>ARGONAUTE1</i> gene	Most stable – NormFinder ( <i>H2B and TUA</i> ) geNorm ( <i>IDH and SAND</i> )	de Almeida et al. 2010
<i>A. hypogaea</i>	<i>GAPDH, UBQ10, ACT7</i> , 60S ribosomal protein L7 ( <i>rpl7</i> ), <i>UBC, EF1α, 14-3-3, adh3, hell</i> (RNA helicase I) and <i>yls8</i> (Yellow leaf specific 8 gene)	<i>Adh3, RPS7</i> and <i>yls8</i> Less stable – <i>14-3-3 and UBQ10</i>	Brand and Hovav, 2010
<i>C. sativus</i>	<i>ACT, EF1α, TUA, UBI-I, PP2A, YLS8, AP47 (CACS), CYC, PDF2</i> (Protein phosphatase 2), <i>Helicase, Expressed protein</i> (At4g33380) and <i>F-box protein</i>	Most stable ( <i>AP47 (CACS), PP2A, F-box protein</i> and <i>EF1α</i> ) and least stable - <i>TUA</i>	Migocka and Papierniak, 2010

**Table 1b:** Microarray based selection of internal controls and their validation in quantitative real-time PCR for target gene normalization in plants

Plant	Microarray based selections of consistent internal reference genes	Validation of selected references through qRT-PCR	Reference
<i>A. thaliana</i>	20 novel stable reference genes were selected from affymetrix ATH1 data and compared with five traditional genes ( <i>UBQ10</i> , <i>ef1a</i> , <i>GADPH</i> , <i>UBC9</i> , <i>ACT2</i> and <i>UBC</i> ) for expression stability and transcription normalization	All novel genes outperformed traditional references, however, among traditional references, <i>UBQ10</i> showed stable expression	Czechowski et al. (2005)
<i>G. max</i>	1. 18 putative soybean reference gene ( <i>cons1-cons18</i> ) selected through affymetrix array hybridization (Total 217 gene identified) 2. <i>SUBI2</i> (D26092) and <i>ACT</i> (AW350943) soybean genes were compared with the new reference genes	Uniformly expressed genes using array : <i>cons4</i> , 6, 7, 8 (Affymetrix) / <i>cons4</i> , 5, 7, and 8 (cDNA array) Reference gene for RT-qPCR : Four genes ( <i>cons4</i> , 6, 7, and 15) were the most stably expressed independent of the data set analyzed During nodulation: ( <i>SUBI2</i> and <i>cons5</i> )	Libault et al. 2008
<i>T. aestivum</i>	1. 32 references genes selected from UniGene EST database of <i>T. aestivum</i> (Total 177 UniGene clusters were identified) 2. Out of the 32, 12 were related to well-known HKGs 3. Cold- and heat-responsive genes( <i>wcor14</i> and <i>TaHSP101B</i> ) were selected for temperature stresses	Stable reference genes <i>Ta54227</i> (Cell division control prot., AAA-superfamily of ATPases) <i>Ta2291</i> (ADP-ribosylation factor) <i>Ta2776</i> (RNase L inhibitor-like protein)	Paolacci et al. 2009
<i>O. sativa</i>	25 novel and six traditional genes ( <i>UBQ5</i> , <i>UBQ10</i> , <i>ACT1</i> , <i>GAPDH</i> , <i>ACT3</i> , $\beta$ - <i>TUB</i> ) were identified from 57 K Affymetrix Genechip Rice whole genome arrays	1. All novel references outperformed traditional references 2. Uniformly expressed internal control genes (Expressed protein, Triosephosphate isomerase, Pre-mRNA-splicing factor SLU7) also showed stable expression in qRT-PCR	Jain, 2009
<i>O. sativa</i>	1. 12 reference genes selected through affymetrix array hybridization (Total 151 gene identified), validated and compared with 14 reference genes ( <i>ACT</i> , <i>ACT1</i> , $\alpha$ - <i>TUB</i> , <i>UBQ</i> , <i>Ubi10</i> , <i>GAPDH</i> , <i>EF1a</i> , <i>TIP41</i> -like, Arabinogalactan protein, <i>EF1d</i> , <i>FtsH</i> protease, Nucleotide tract-binding Protein, <i>AP-2</i> complex subunit, endo-1,4-beta-glucanase ) from the previous studies 2. Compared the 12 reference genes to 3 heat or cold responsive genes [Apetala type transcription factor ( <i>AP2</i> ), a heat shock responsive factor ( <i>HSF-82</i> ) and alternative oxidase ( <i>AOX</i> )] over time under i) cold or ii) heat conditions respectively	1. All 12 genes outperformed previously used references 2. Protein kinase-1 and a nucleic acid binding protein showed stable, moderate expression levels across the stress microarray set 3. Tumor protein homolog and translation initiation factor <i>SUI1</i> showed stable expression across the developmental and entire microarray sets respectively 4. Nucleic acid binding protein showed stable expression under qRT-PCR followed by protein kinase	Narsai et al. 2010
Chinese Spring Wheat	1. 32 genes were selected from Wheat Genome arrays 2. 15 genes finally evaluated as internal control, out of which seven novel genes compared with 13 traditional references	Most stable: <i>Ta.27922.1.S1_at</i> (cyclin), <i>Ta.10105.1.S1_at</i> (heterogeneous nuclear ribonucleoprotein) and <i>Ta.14126.1.S1_at</i> (scaffold-associated regions) Note: Novel genes outperformed traditional references	Long et al. 2010