

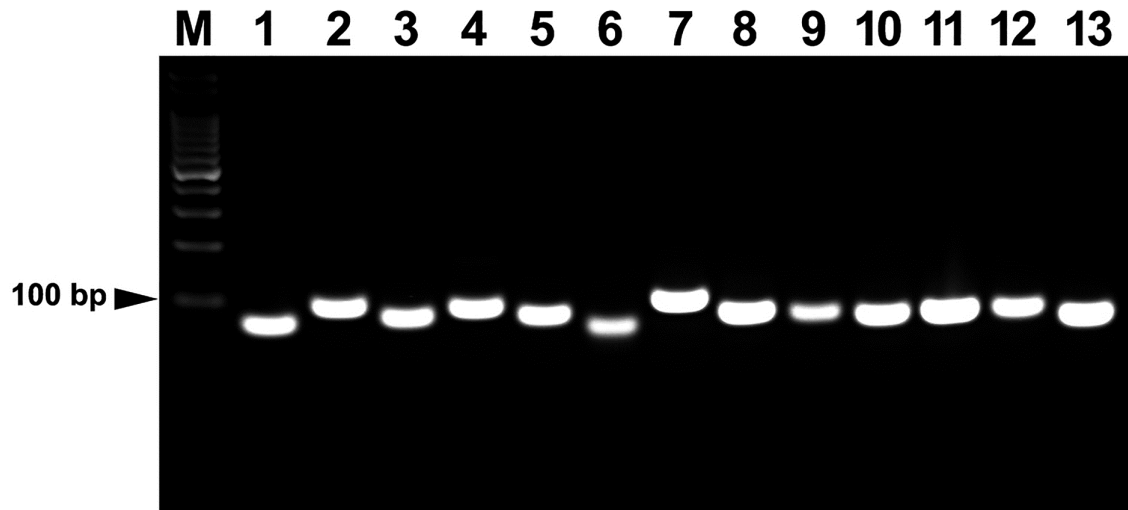
**Selection of reliable reference genes for RT-qPCR analysis during developmental stages and abiotic stress in *Setaria viridis***

Polyana Kelly Martins<sup>1</sup>, Valéria Mafra<sup>2</sup>, Wagner Rodrigo de Souza<sup>1</sup>, Ana Paula Ribeiro<sup>1</sup>, Felipe Vinecky<sup>1</sup>, Marcos Fernando Basso<sup>1</sup>, Bárbara Andrade Dias Brito da Cunha<sup>1</sup>, Adilson Kenji Kobayashi<sup>1</sup> and Hugo Bruno Correa Molinari<sup>1\*</sup>

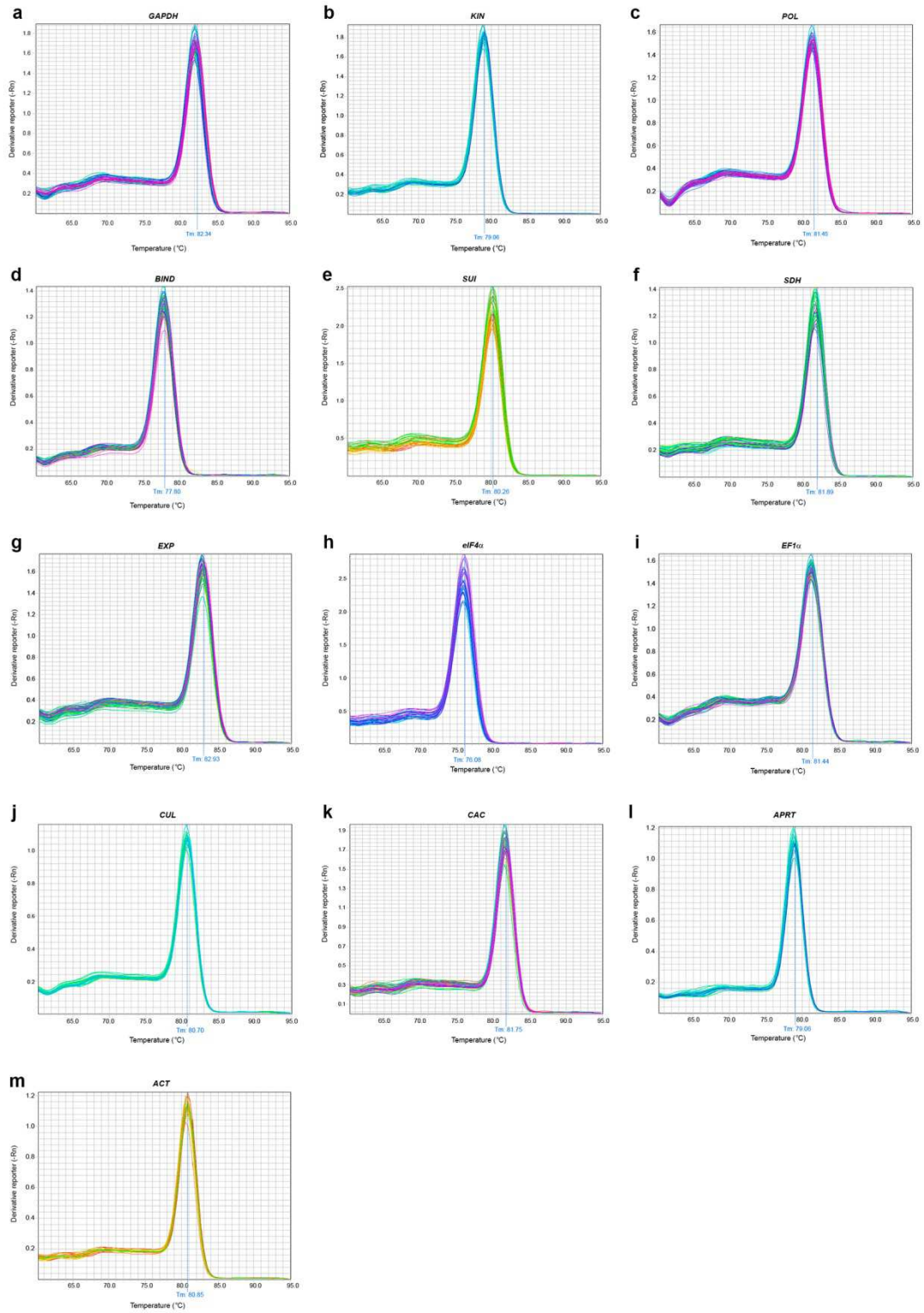
<sup>1</sup>Genetics and Biotechnology Laboratory, Embrapa Agroenergy (CNPAE), Brasília, DF, 70770-901, Brazil.

<sup>2</sup>Brazilian Bioethanol Science and Technology Laboratory/Brazilian Center of Research in Energy and Materials, Campinas, SP, 13083-100, Brazil.

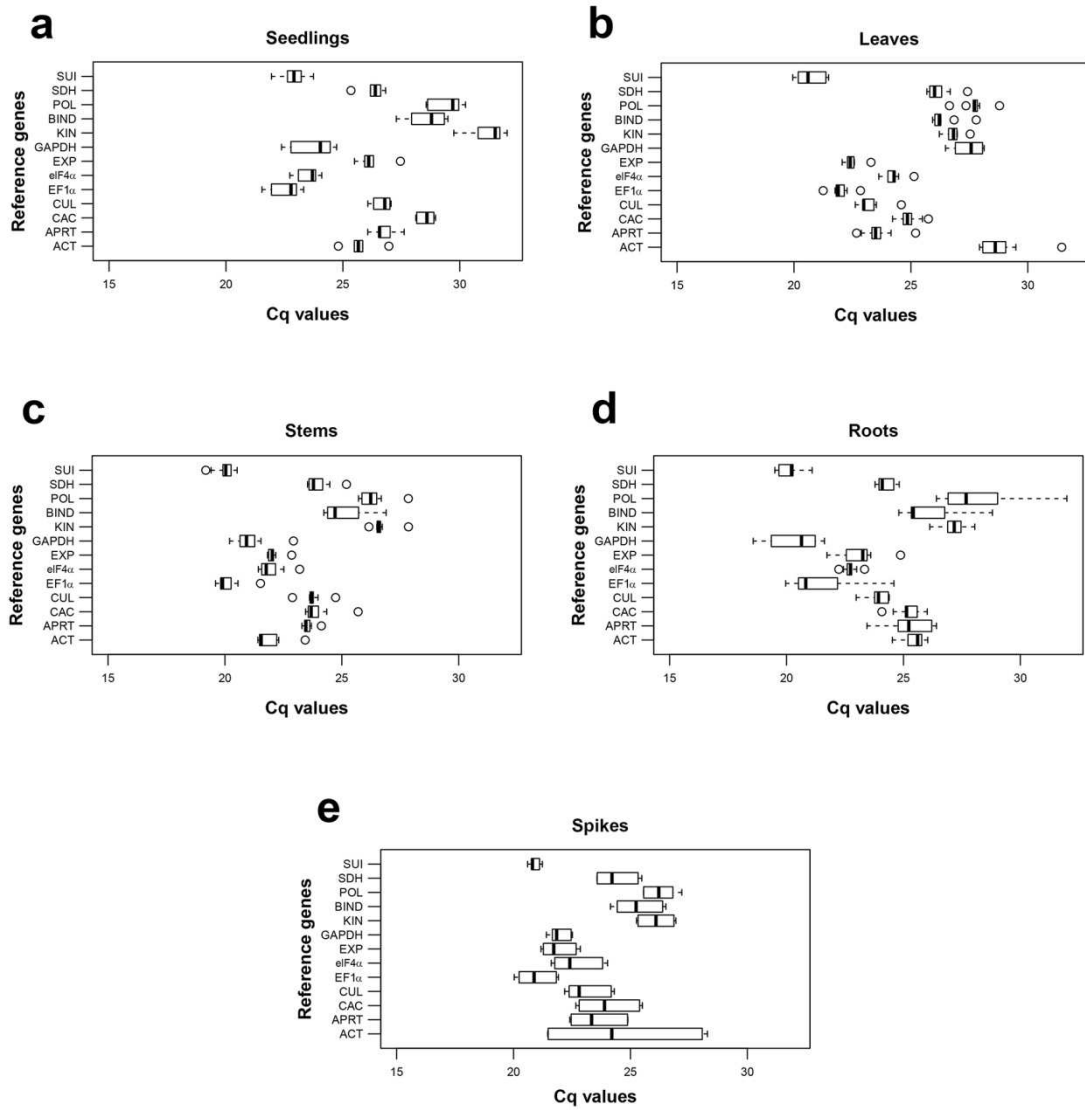
\*Corresponding author: Phone: +55 61 3448 2307; +55 61 3448 1589. E-mail address: hugo.molinari@embrapa.br



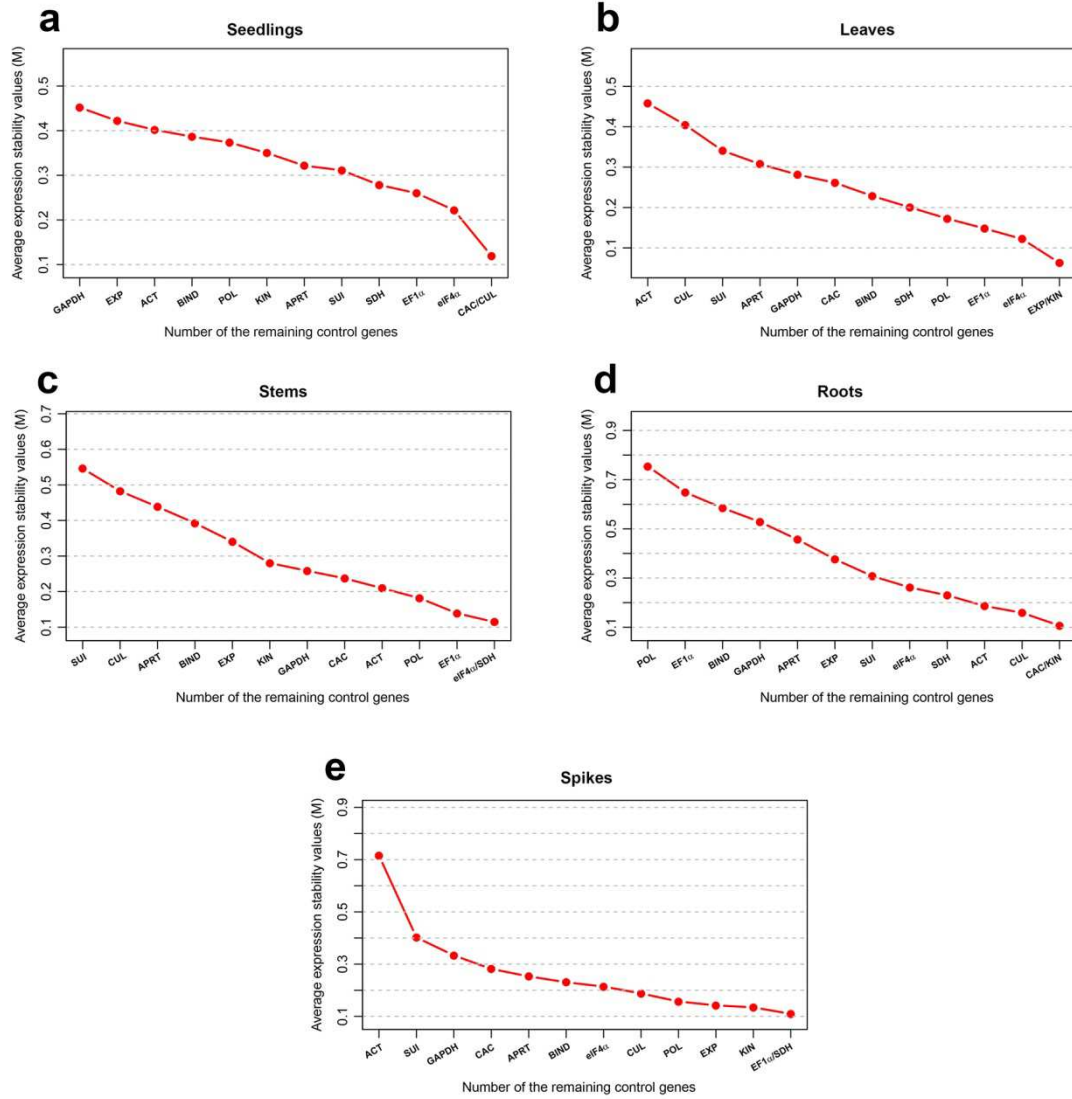
**Figure S1:** RT-qPCR amplification specificity of the 13 reference genes. Amplification fragments were separated by 2% agarose gel electrophoresis. 1 – *eIF4 $\alpha$* , 2 – *ACT*, 3 – *SDH*, 4 – *CAC*, 5 – *CUL*, 6 – *POL*, 7 – *APRT*, 8 – *EXP*, 9 – *BIND*, 10 – *KIN*, 11 – *GAPDH*, 12 – *SUI* and 13 – *EF1 $\alpha$* .



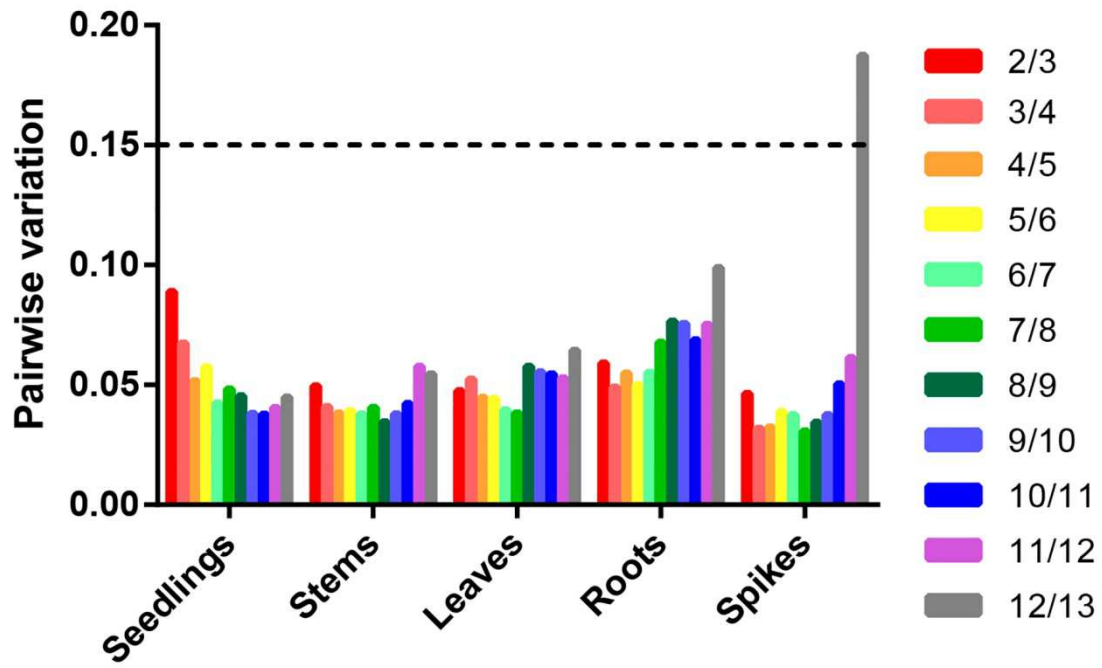
**Figure S2:** Dissociation curve data for the 13 reference genes tested. **(a)** *GAPDH*; **(b)** *KIN*; **(c)** *POL*; **(d)** *BIND*; **(e)** *SUI*; **(f)** *SDH*; **(g)** *EXP*; **(h)** *eIF4 $\alpha$* ; **(i)** *EF1 $\alpha$* ; **(j)** *CUL*; **(k)** *CAC*; **(l)** *APRT* and **(m)** *ACT*.



**Figure S3:** Expression level of reference genes tested in different tissues/organs. (a) Seedlings; (b) Leaves; (c) Stems; (d) Roots and (e) Spikes.



**Figure S4:** Average expression stability values (M) calculated by geNorm. A lower value of average expression stability (M) indicates most stable expression. **(a)** Seedlings; **(b)** Leaves; **(c)** Stems; **(d)** Roots and **(e)** Spikes.



**Figure S5:** Pairwise variation (V) to define the optimal number of reference genes required to a more reliable normalization to seedlings, stems, leaves, roots and spikes.

**Table S1:** Information on reference and target genes used for qRT-PCR analysis

Gene symbol	Description	Source sequence ID	<i>S. viridis</i> Phytozome ID	E-value	<i>S. viridis</i> identity %	Primer sequences (5'-3')	T <sub>m</sub> (°C)	Amplicon size (bp)	PCR Efficiency (%)	Reference
<i>ACT</i>	<i>Actin</i>	Si026509m	Sevir.8G042300	0	100	F: GCACCACCTGAGAGGAAAATATAG R: CTCATCGTACTCCGCCTTTG	72.1 70.2	102	92	[29]
<i>eIF4a</i>	Eukaryotic initiation factor 4-alpha	CA275432.1	Sevir.1G088000	0	100	F: GTCTGCTAAGGTGCTGGATAAA R: ACCACTCCTCCAGAACATAGA	69.0 69.1	78	93	[27]
<i>CUL</i>	Cullin	CF574093.1	Sevir.3G038900	1.6e-121	93.1	F: TCTCATCACGAGGGACTACTT R: CTTGCCAACAACCACCAATC	69.2 68.0	85	96	[27]
<i>APRT</i>	Anthranilate phosphoribosyl transferase	CA089592.1	Sevir.2G326600	0	100	F: GGCAACGGCTATGTTTCTAATA R: CCTCATCCAGAAGAACCCAAG	69.1 71.0	103	93	[28]
<i>CAC</i>	Clathrin adaptor complex	CA203604.1	Sevir.1G284400	2.3e-54	100	F: CTGCTTCTGGTCTTCGTGTT R: GTATGATCCTGCTCTCGTGATG	68.2 71.1	98	92	[27]
<i>GAPDH</i>	Glyceraldehyde 3-phosphate dehydrogenase	LOC_Os02g38920	Sevir.1G225700	2.1e-64	100	F: TGACAACAGGTCCAGCATCTTT R: CGTACCAGGACACAAGCTTCAC	65.8 65.8	80	91	[31]
<i>SUI</i>	Translation factor SUI1	LOC_Os07g34589	Sevir.2G348300	2e-90	100	F: CCAGAGCTTGGACAGGTCATTC R: ACAATGCCAGCCTGGACAA	67.1 66.8	80	90	[31]
<i>KIN</i>	Protein kinase	LOC_Os06g48970	Sevir.4G252700	5.8e-78	99.2	F: AAGTCCACTCGACCTTCAATCC R: CTCAAGCGAACCGAATGAAAC	65.4 65.7	80	94	[31]
<i>BIND</i>	RNA-binding protein	LOC_Os03g46770	Sevir.9G090900	3.5e-89	71.9	F: CTCGCGGTTTCGGCTTT R: CCAAGTCCAGTCCATTCATTCC	66.4 66.6	81	91	[31]
<i>EF1a</i>	Elongation Factor 1-alpha	Si022039m	Sevir.3G272400	0	96.1	F: TGGTATGCTTGTCACCTTTGGT R: CTCGTGGTGCATCTCAACTGA	65.4 66.5	70	92	[29]
<i>POL</i>	RNA polymerase II	Si033113m	Sevir.2G148400	0	92.9	F: TTGAGGCTTCGTGCCATTG R: CGGTAGCAGCTGGTGACCAT	67.3 67.5	70	93	[29]
<i>SDH</i>	Succinate dehydrogenase	Bradi1g26050	Sevir.7G038300	8.9e-83	100	F: ACAGTTCAGCGCTACGTTCT R: GGTGGCTAGAGGGACGGATA	60.4 64.5	85	90	[30]
<i>EXP</i>	Expressed protein	LOC_Os07g02340	Sevir.9G362700	3.6e-15	47.8	F: GGAACGCCGAGAAGAACAAG R: ACATACTTTGCACTGGATGCTCAT	66.0 65.6	83	92	[31]
<i>GOLS2</i>	Glycosyl transferase	AT1G56600	Sevir.2G450900	2.6e-107	99.7	F: CGAGGACATCAAGATGCTGGTGAG R: TCCAGGCTCTCGTCGTTGAAGATG	71.0 72.4	62	95	[35]
<i>P5CS</i>	$\Delta^1$ -pyrroline-5-carboxylate synthase	AT2G39800	Sevir.5G386300	6.4e-43	100	F: TTGGTCTAGGTGCAGAGGTTGG R: AGCCCATCAACACCAACAGGTC	67.4 69.3	73	100	[36]
<i>SuSy</i>	Sucrose Synthase	AF263384	Sevir.4G039300	9.9e-149	99.9	F: CTGACTGGTGTGTACGGATTC R: CTTGCCAGGCTACGGTATTT	71.0 68.0	107	91	[57]

**Table S2.** *Setaria viridis* reference genes ranked according to expression stability as determined by geNorm.

Ranking	Seedlings		Leaf		Stem		Root		Spike	
	geNorm	Stability value	geNorm	Stability value	geNorm	Stability value	geNorm	Stability value	geNorm	Stability value
1	<i>CAC</i>	0.12	<i>EXP</i>	0.11	<i>eIF4a</i>	0.06	<i>CAC</i>	0.11	<i>CAC</i>	0.11
2	<i>CUL</i>	0.12	<i>KIN</i>	0.11	<i>SDH</i>	0.06	<i>KIN</i>	0.11	<i>KIN</i>	0.11
3	<i>eIF4a</i>	0.22	<i>eIF4a</i>	0.14	<i>EF1a</i>	0.12	<i>CUL</i>	0.16	<i>CUL</i>	0.16
4	<i>EF1a</i>	0.26	<i>EF1a</i>	0.18	<i>POL</i>	0.15	<i>ACT</i>	0.19	<i>ACT</i>	0.19
5	<i>SDH</i>	0.28	<i>POL</i>	0.21	<i>ACT</i>	0.17	<i>SDH</i>	0.23	<i>SDH</i>	0.23
6	<i>SUI</i>	0.31	<i>SDH</i>	0.24	<i>CAC</i>	0.20	<i>eIF4a</i>	0.26	<i>eIF4a</i>	0.26
7	<i>APRT</i>	0.32	<i>BIND</i>	0.26	<i>GAPDH</i>	0.23	<i>SUI</i>	0.31	<i>SUI</i>	0.31
8	<i>KIN</i>	0.35	<i>CAC</i>	0.28	<i>KIN</i>	0.26	<i>EXP</i>	0.38	<i>EXP</i>	0.38
9	<i>POL</i>	0.37	<i>GAPDH</i>	0.34	<i>EXP</i>	0.28	<i>APRT</i>	0.46	<i>APRT</i>	0.46
10	<i>BIND</i>	0.39	<i>APRT</i>	0.39	<i>BIND</i>	0.31	<i>GAPDH</i>	0.53	<i>GAPDH</i>	0.53
11	<i>ACT</i>	0.40	<i>SUI</i>	0.44	<i>APRT</i>	0.34	<i>BIND</i>	0.58	<i>BIND</i>	0.58
12	<i>EXP</i>	0.42	<i>CUL</i>	0.48	<i>CUL</i>	0.40	<i>EF1a</i>	0.65	<i>EF1a</i>	0.65
13	<i>GAPDH</i>	0.45	<i>ACT</i>	0.55	<i>SUI</i>	0.46	<i>POL</i>	0.75	<i>POL</i>	0.75
<b>Best pair</b>	<i>CAC/ CUL</i>		<i>EXP/ KIN</i>		<i>eIF4a/ SDH</i>		<i>CAC/ KIN</i>		<i>CAC/ KIN</i>	

**Table S3.** *Setaria viridis* reference genes ranked according to expression stability as determined by NormFinder.

Ranking	Seedlings		Leaf		Stem		Root		Spike	
	Norm Finder	Stability value	Norm Finder	Stability value	Norm Finder	Stability value	Norm Finder	Stability value	Norm Finder	Stability value
1	<i>SDH</i>	0.01	<i>EXP</i>	-0.003	<i>SDH</i>	0.004	<i>EXP</i>	0.03	<i>eIF4a</i>	-0.02
2	<i>EF1a</i>	0.01	<i>KIN</i>	0.002	<i>eIF4a</i>	0.007	<i>ACT</i>	0.05	<i>BIND</i>	-0.01
3	<i>eIF4a</i>	0.02	<i>eIF4a</i>	0.004	<i>EF1a</i>	0.009	<i>CUL</i>	0.06	<i>SDH</i>	-0.01
4	<i>CAC</i>	0.03	<i>SDH</i>	0.011	<i>EXP</i>	0.015	<i>CAC</i>	0.06	<i>CUL</i>	-0.01
5	<i>CUL</i>	0.04	<i>BIND</i>	0.021	<i>KIN</i>	0.021	<i>KIN</i>	0.07	<i>APRT</i>	-0.01
6	<i>SUI</i>	0.04	<i>EF1a</i>	0.024	<i>POL</i>	0.025	<i>SDH</i>	0.07	<i>EF1a</i>	-0.01
7	<i>KIN</i>	0.04	<i>POL</i>	0.028	<i>ACT</i>	0.026	<i>eIF4a</i>	0.10	<i>KIN</i>	0.02
8	<i>APRT</i>	0.05	<i>CAC</i>	0.040	<i>CAC</i>	0.043	<i>SUI</i>	0.11	<i>CAC</i>	0.02
9	<i>BIND</i>	0.07	<i>GAPDH</i>	0.137	<i>GAPDH</i>	0.059	<i>BIND</i>	0.15	<i>EXP</i>	0.02
10	<i>POL</i>	0.07	<i>APRT</i>	0.167	<i>APRT</i>	0.061	<i>APRT</i>	0.17	<i>POL</i>	0.04
11	<i>ACT</i>	0.08	<i>SUI</i>	0.180	<i>BIND</i>	0.118	<i>EF1a</i>	0.29	<i>GAPDH</i>	0.20
12	<i>EXP</i>	0.13	<i>CUL</i>	0.182	<i>CUL</i>	0.200	<i>GAPDH</i>	0.30	<i>SUI</i>	0.40
13	<i>GAPDH</i>	0.16	<i>ACT</i>	0.331	<i>SUI</i>	0.239	<i>POL</i>	0.78	<i>ACT</i>	2.85
<b>Best pair</b>	<i>SDH/ EF1a</i>		<i>SDH/ KIN</i>		<i>eIF4a/ EXP</i>		<i>CUL/ KIN</i>		<i>eIF4a/ APRT</i>	



