

Supplementary material of the manuscript published in Vitis 55, 165–171 (2016):

Identification, characterization and expression analysis of ERF transcription factor *VviERF073* and standardization of stable reference genes under salt stress in grape

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Supplementary Table 1

List of *Arabidopsis* homologous genes interacting with VviERF073 (GSVIVP00028041001)

S. No.	<i>Vitis vinifera</i> genes/protein interacting with VviERF073	Homologous gene in <i>Arabidopsis thaliana</i>	Biological role
1	GSVIVG000033487001	XTR7 AT4G14130.1-P	XTR7 (Xyloglucan Endotransglycosylase 7); Catalyzes xyloglucan endohydrolysis and/ or endotransglycosylation
2	GSVIVG00014541001	AtCKX6 AT1G75450.1-P	CKX5 (Cytokinin Oxidase 5); cytokinin dehydrogenase
3	GSVIVG00028044001	ERS1 AT2G40940.1-P	ERS1 (Ethylene Response Sensor 1); ethylene binding / protein histidine kinase/ receptor
4	GSVIVG00016760001	MBF1C AT3G24500.1-P	MBF1C (Multiprotein Bridging Factor 1C); DNA binding / transcription coactivator
5	GSVIVG00002180001	EIN 4 AT3G04580.1-P	EIN4 (Ethylene Insensitive 4); ethylene binding / receptor
6	GSVIVG00037194001	AT2G46690.1-P	Auxin-responsive family protein; auxin-responsive family protein
7	GSVIVG00034466001	IAA19 AT3G15540.1-P	IAA19 (Indole-3-Acetic Acid Inducible 19); transcription factor
8	GSVIVG00031238001	AT2G36690.1-P	Oxidoreductase, 2OG-Fe(II) oxygenase family protein
9	GSVIVG00022326001	AT1G71090.1-P	Auxin efflux carrier family protein
10	GSVIVG00021917001	AT1G76680.2-P	OPR1; 12-oxophytodienoate reductase; Encodes a member of an alpha/beta barrel fold family of FMN-containing oxidoreductases
11	GSVIVG00019364001	BRI1 AT4G39400.1-P	BRI1 (Brassinosteroid Insensitive 1); kinase
12	RD22-B GSVIVG00016747001	RD22 AT5G25610.1-P	RD22; nutrient reservoir; responsive to dehydration 22 (RD22) mediated by ABA
13	GSVIVG00031249001/ GSVIVG00031237001	AT2G44800/ AT4G10490	Oxidoreductase, 2OG-Fe(II) oxygenase family protein
14	GSVIVG00028908001/ GSVIVG00021916001/ GSVIVG00021911001/ GSVIVG00021908001	OPR2	Encodes one of two closely related 12-oxophytodienoic acid reductases
15	GSVIVG00023625001	GID1B	(GA INSENSITIVE DWARF1B); encodes a gibberellin (GA) receptor
16	GSVIVG00019408001	CPR5	CPR5 (Constitutive Expression of PR Genes 5); Regulator of expression of pathogenesis-related (PR) genes

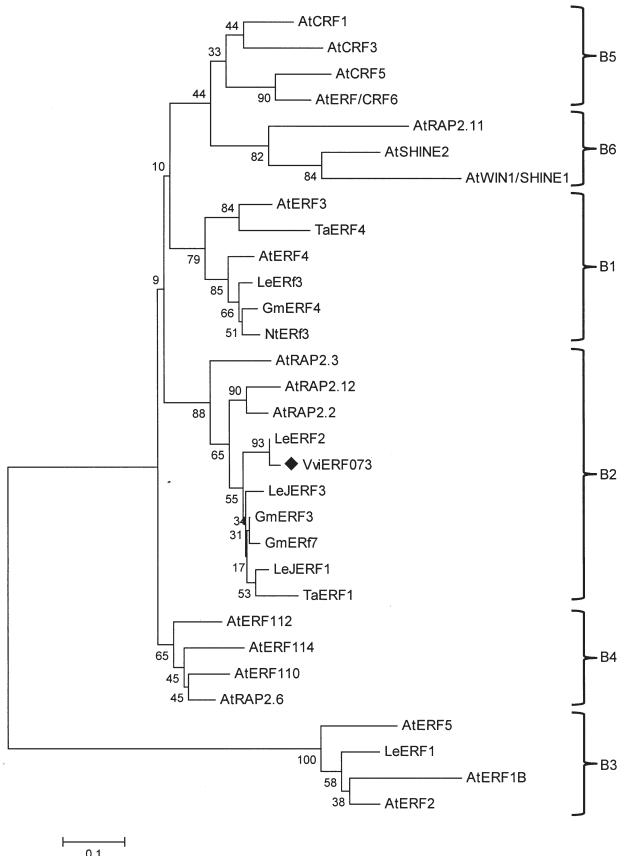
Supplementary Table 2

Details of candidate reference genes

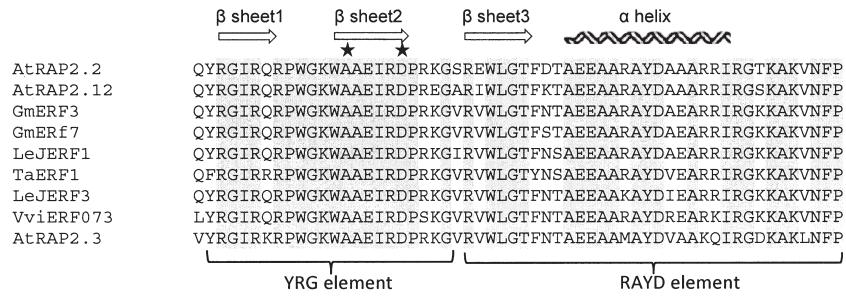
Gene name (abbreviation)	Primer sequence (5'-3')	PCR Efficiency	R ²
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	F: TTC TCG TTG AGG GCT ATT CCA R: CCA CAG ACT TCA TCG GTG ACA	0.93	0.99
Actin 7/actin 2 (Actin)	F: CTT GCA TCC CTC AGC ACC TT R: TCC TGT GGA CAA TGG ATG GA	0.92	0.99
Aquaporin (AqPO)	F: TCC GCC AAG GAC TAT CAT GAC R: CGC AAT CAG AGC CCT GTA GAA	0.98	0.99
Elongation factor 1 α (EF1 α)	F: GAA CTG GGT GCT TGA TAG GC R: AAC CAA AAT ATC CGG AGT AAA AGA	1.0	0.99
Tubulin α 3/ α 5 chain (Tubulin)	F: CAG CCA GAT CTT CAC GAG CTT R: GTT CTC GCG CAT TGA CCA TA	1.0	0.98
SAND family protein (SAND)	F: CAA CAT CCT TTA CCC ATT GAC AGA R: GCA TTT GAT CCA CTT GCA GAT AAG	1.05	0.98
Sucrose transporter (Sutra)	F: GGA TAA CTT CCC TGC CTC AAT GA R: TTC TTG TAG CAG CTG AGA GGA TCA	0.96	0.99
Ubiquitine (UBI)	F: AGT AGA TGA CTG GAT TGG AGG T R: GAG TAT CAA AAC AAA AGC ATC G	0.97	0.99

1 TTTCTTTCTGTGATCGAAGTCATTGAAAGACTGAAGA
 40 ATGTGTGGAGGGTGTATCATCTCTGATTTCATACCTCGCAACCGGAACCGCCGCGACCCA
 1 M C G G A I I S D F I P R N R N R R D P
 100 GCCCCAGACCTCTGGCCGACTCTTCCTCGCTAAACCCGATGGCTGTAATACGATTG
 21 A P D L W P S F F E A K P D G C E Y D L
 160 GGCGCGCTTAGCCAGAAGGGGCTCTCAATCTGAAAAGGTCTAACCCATTGGTGTGAT
 41 G R F S Q K G L P N L K R S Q P I L D D
 220 GAGCCAGAAGTGAAAGCCGGCTAAAGGGGTGAGGAAGAATCTGACAGGGGATCCGGCAG
 61 E P E V K P A G K R V R K N L Y R G I R Q
 280 CGTCCTTGGGAAATGGGCCGCTGAGATTGCTGATCCCAGTAAAGGGGTTGGGTTGG
 81 R P W G K W A A E I R D P S K G V R V W
 340 CTGGGACCTTCAACACTCGCGGAAGAAGCTCGCGAGAGCTACGACAGGGAGGCTGGAAA
 101 L G T F N T A E E A A R A Y D R E A R K
 400 ATTCCGGGGAAAGAACAAAGGTAAACCTCCCAATGAAGACGATGACTACACTGGAAAT
 121 I R G K K A K V N F P N E D D D Y T E N
 460 CACCAAATACCGTGCTTACAACCTCGCTGGAACCTCAATCTTCTATTGTCAGCCC
 141 H Q N H R A L P T R W N S N P S I C Q P
 520 TATACCCCCAATTTCTCAAAAGTTGGTTTGTGATCACTGAAACCAAGATTGGCA
 161 Y T P N F S K S L G F A D H L N Q I G A
 580 TTCCCCCTCAATGGGTCAACACCGTTGGTGTATGAACGCTAACCGGTTGCTGTCACA
 181 F P S N G F T V G A M N A N P V A V T
 640 ACCGAAAGTGAATAACCGGCTCTGATTGCGAGTGTGATCCTCTCTGGATTGGAAT
 201 T E V K Y G S D S E S V Y P S S G L L N
 700 TGCAATCAGAAAGCTGTGTTGGAAAGTGAAGAAATCAGAAAGAGAACCAAACAGAGACT
 221 C N Q K A C V G K V K N Q K E N Q T E T
 760 GTGACCGGAAGCTGAGAAGGGAAATGGAAAGTGCAGAACGGTGTGGAGGAGCTAATGGCG
 241 V T E A E K E E M E V Q K L S E E L M A
 820 TATGAGTCGGTCAAGTTCTCATCAGATCCCCTATCTCGACGGCAACTCAGCCGGTT
 261 Y E S V M K F Y Q I P Y L D G N S A A V
 880 CCCAACGCTGTCAGGAAATCTGGTGTGCTGGTGTGCTGCAATGAAAGCTTGG
 281 P N A A Q E N A G A C G G A A M E A L W
 940 AGCTTTGATGAATTGTCAGTTGCTCTCAACCACCTCACGTGCTCTATAA
 301 S F D E F A V A P Q P T S A A L *

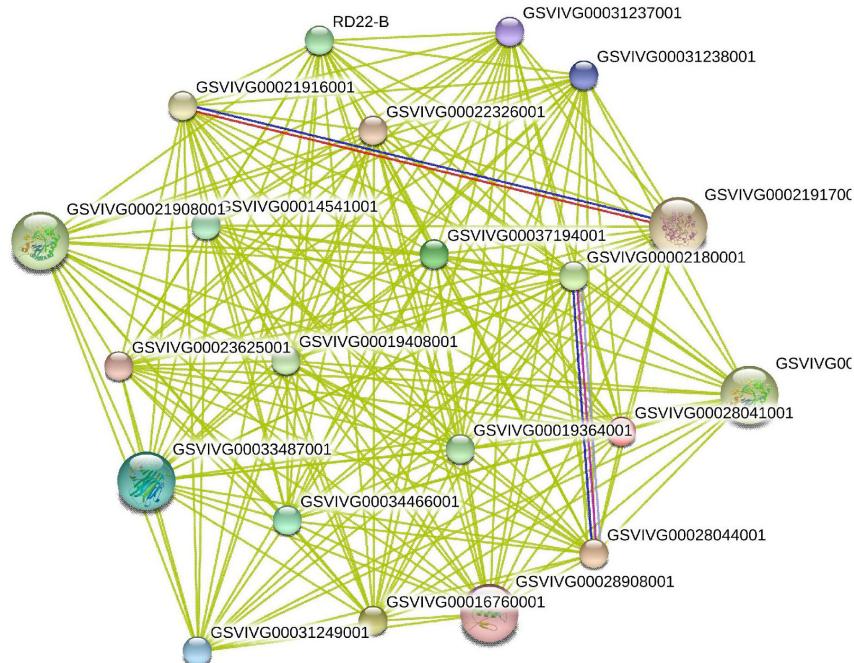
Supplementary Fig. 1: DNA sequence and deduced polypeptide sequence of *VviERF073*. The conserved ERF domain is shown with shaded background. Conserved N terminal sequence characteristic of B2 group is underlined.



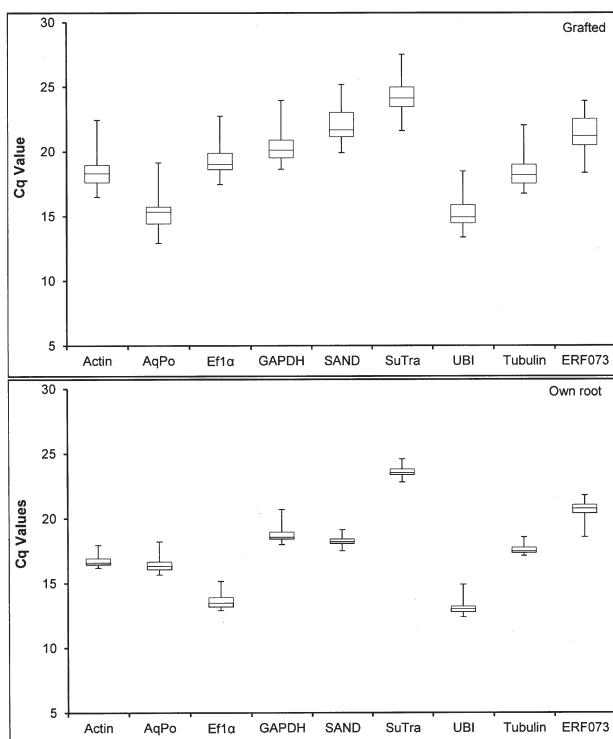
Supplementary Fig. 2: Phylogeny analysis based on full length protein sequences. *VviERF073* grouped with ERF proteins of B2 group.



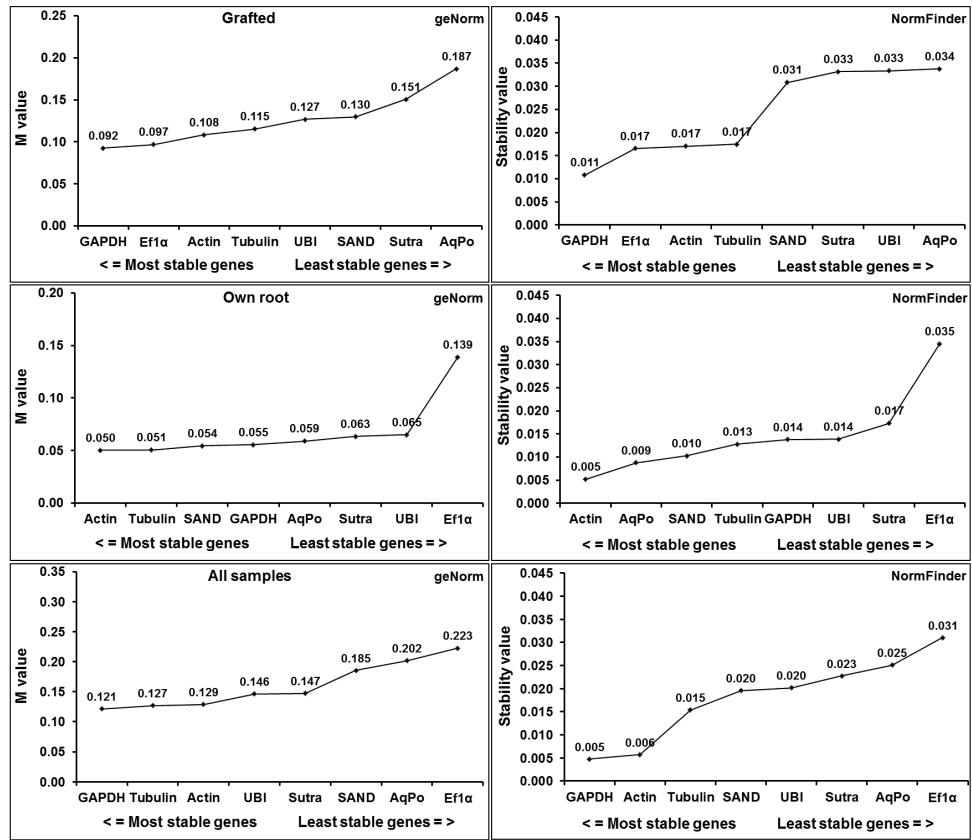
Supplementary Fig. 3: The conserved AP2/ERF domain of the B2 group ERF proteins. ★ indicates the 14th and 19th amino acid residues.



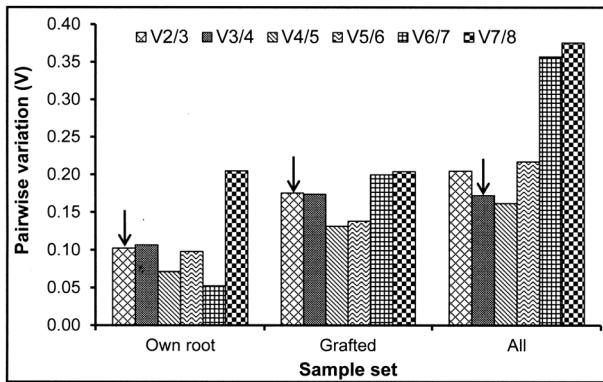
Supplementary Fig. 4: Interaction analysis of *VviERF073* with other proteins using STRING9.1.



Supplementary Fig. 5: Expression levels (Cq) of different candidate reference genes in leaves of 'Thompson Seedless' raised on grafted and own root. The line across the box depicts the median. The box indicates the 25th and 75th percentiles, and whisker caps represent the maximum and minimum values. The higher box and whisker height indicates greater variation.



Supplementary Fig. 6: Expression stability and ranking of reference genes in different sample sets based on geNorm and NormFinder.



Supplementary Fig. 7: Pairwise variation of reference genes in different sample sets. Arrow indicates the optimal number of genes selected for data normalization.